IBRO Reports 9 (2020) 233–240

Contents lists available at ScienceDirect

IBRO Reports

journal homepage: www.journals.elsevier.com/ibro-reports

Research Paper

Reduced brain fractalkine-CX3CR1 signaling is involved in the impaired cognition of streptozotocin-treated mice

Namiko Kawamura [a,*], Goro Katsuura [a], Nobuko Yamada-Goto [b,c], Ela Novianti [a], Akio Inui [d], Akihiro Asakawa [a]

[a] Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[b] Health Center, Keio University, Japan
[c] Division of Endocrinology, Metabolism and Nephrology, Department of Internal Medicine, Keio University, School of Medicine, Japan
[d] Pharmacological Department of Herbal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

ARTICLE INFO

Keywords:
Diabetes
Streptozotocin
Fractalkine
CX3CR1
Memory
Mice

ABSTRACT

Patients with diabetes mellitus are predisposed to cognitive impairment. Fractalkine-CX3CR1 in the brain signaling represents a primary neuron-microglia inter-regulatory system for several brain functions including learning and memory processes. The present study addressed whether fractalkine-CX3CR1 signaling in the hippocampus contributes to the cognitive deficits observed in streptozotocin (STZ)-treated mice. Our results showed that STZ-treated mice exhibited significant cognitive deficits in the Y-maze test, and a decrease in fractalkine and CX3CR1 levels in the hippocampus. Moreover, intracerebroventricular injection of the CX3CR1 antagonist 18a in normal mice induced significant cognitive deficits in the Y-maze test. STZ-treated mice showed a significant increase in plasma corticosterone levels and a decrease in plasma and hippocampal levels of insulin-like growth factor-1 (IGF-1). Therefore, we examined the effects of corticosterone and IGF-1 on regulation of fractalkine and CX3CR1 expression. Dexamethasone (DEX) application significantly decreased the mRNA expression of fractalkine in primary neuron and astrocyte cultures, and of CX3CR1 in primary microglia cultures. On the other hand, IGF-1 application significantly increased the mRNA expression of fractalkine in primary neuron cultures and CX3CR1 in primary microglia cultures. In addition, administration of DEX and the IGF-1 receptor tyrosine kinase inhibitor picropodophyllin significantly reduced the mRNA expression of fractalkine and CX3CR1 in the hippocampus. These findings indicate that impaired cognition in STZ-treated mice is associated with reduced fractalkine-CX3CR1 signaling in the hippocampus which may be induced by an increase in corticosterone and a decrease in IGF-1.

1. Introduction

Fractalkine secreted from neurons was recently reported to be involved in the regulation of several functions of the central nervous system (CNS) (Goazigo et al., 2013; Maciejewski-Lenoir et al., 1999; Nishiyori et al., 1998). Astrocytes in the brain also synthesize fractalkine (Yoshida et al., 2001). In mouse brain, fractalkine mRNA levels are high in the cortex, hippocampus and striatum; intermediate in the olfactory bulb, thalamus, hypothalamus and brainstem; and low in the cerebellum (Maciejewski-Lenoir et al., 1999; Nishiyori et al., 1998). Fractalkine binds to the CX3C chemokine receptor 1 (CX3CR1), which is mainly expressed in microglia (Imai et al., 1997; Maciejewski-Lenoir et al., 1999; Nishiyori et al., 1998). Fractalkine-CX3CR1 signaling represents a primary neuron-microglia inter-regulatory system that is important for synaptic plasticity and function in the brain (Goazigo et al., 2013). Recent evidence indicates that fractalkine-CX3CR1 signaling plays an important role in regulating the formation of long-term potentiation (LTP) in the hippocampus and behavioral learning and memory processes (Rogers et al., 2011; Sheridan et al., 2014). LTP, defined as an activity-dependent, prolonged enhancement of synaptic strength, in the

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS, central nervous system; CX3CR1, CX3C chemokine receptor 1; DEX, dexamethasone; DM, diabetes mellitus; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IGF-1, insulin-like growth factor-1; LTP, long-term potentiation; NMDA, N-methyl-D-aspartate; PPP, picropodophyllin; STZ, streptozotocin.

* Corresponding author.
E-mail address: nkawamu@m3.kufm.kagoshima-u.ac.jp (N. Kawamura).

https://doi.org/10.1016/j.ibror.2020.09.002
Received 26 June 2020; Accepted 9 September 2020
Available online 15 September 2020

hippocampal CA1 region is considered to be a form of synaptic plasticity for the cellular mechanisms of learning and memory, and is predominately regulated by the glutamatergic system (Neves et al., 2008). CX3CR1-deficient mice exhibit cognitive deficits in different types of learning and memory tasks, such as fear-conditioning and water maze tests, in parallel with impaired LTP (Rogers et al., 2011).

Diabetes mellitus (DM) is a common metabolic disorder, characterized by glucose intolerance. Epidemiologic studies have demonstrated that both type 1 and type 2 DM patients have a predisposition for several dysfunctions of the CNS, such as cognitive impairment and depression, compared with non-diabetic patients (Biessels et al., 2008; McCall, 1992). Our previous study demonstrated that diet-induced obese mice fed a high-fat diet exhibit significant impairment of fear conditioning responses which are dependent on the hippocampus and amygdala (Yamada-Goto et al., 2012). Streptozotocin (STZ)-treated animals as a model of type 1 DM exhibit impaired learning and memory in several learning behavioral tests, such as the Y-maze, water maze, complex maze and passive avoidance (Biessels et al., 1996; Molteni et al., 2002; Wu et al., 2004). Moreover, electrophysiological studies revealed impaired expression of LTP in the hippocampus of STZ-treated animals (Kamal et al., 1999, 2000).

In the present study, we examined the possible contribution of fractalkine-CX3CR1 signaling in the hippocampus to the impaired cognition observed in STZ-treated mice. Our findings revealed that impaired cognition in STZ-treated mice is associated with decreased fractalkine-CX3CR1 signaling in the hippocampus which may be induced by an increase in plasma corticosterone levels and a decrease in plasma and hippocampal insulin-like growth factor-1 (IGF-1) levels.

2. Material and methods

2.1. Animals

Male C57BL/6 J mice (6 weeks old) were obtained from CLEA Japan, Inc. (Tokyo, Japan) and housed in plastic cages under a 12:12 h light/dark cycle (lights turned on at 07.00 h) at room temperature (23 ± 1 °C). The animals had *ad libitum* access to water and food (CE-2; CLEA Japan, Inc.). All experiments were performed in accordance with the guidelines established by the Institute of Laboratory Animal Science Research Support Center at Kagoshima University and approved by the Kagoshima University Institutional Animal Care and Use Committee (protocol nos. MD18079 and MD18080), and in accordance with the guidelines established by the United States National Institutes of Health Guide for the care and use of laboratory animals (NIH publication No. 80-23, revised in 1996). Every effort was made to optimize the comfort of the animals and to minimize their use.

2.2. STZ-treated mice

Mice (10–12 weeks old) were given intraperitoneal injections of a single dose of STZ (200 mg/kg body weight, Merck KGaA, Darmstadt, Germany). STZ was dissolved in 10 mM chilled sodium citrate buffer (pH 4.0) just before injection. Control mice were given intraperitoneal injections of an equal volume of sodium citrate buffer (10 ml/kg body weight). Two weeks after the injection, we evaluated them in the Y-maze test and measured their body weight before the mice were killed by an overdose of isoflurane (Abbott Japan, Tokyo, Japan) for collection of blood samples and brain region.

2.3. Analysis of metabolic parameters and sampling of the brain region

Blood samples were collected from the retroorbital vein under isoflurane anesthesia and immediately transferred to tubes containing ethylenediaminetetraacetic acid (EDTA; 10 μl of 0.2 M EDTA/tube) and aprotinin (0.1 mg/tube, Merck KGaA). The blood samples were centrifuged 3000×g for 5 min at 4 °C, and the plasma was separated and stored at −80 °C until assayed. After blood collection, the mice were killed by decapitation. The brain was rapidly removed from the skull and placed on an ice-cooled paraffin plate for dissection of the hippocampus as previously described (Nakao et al., 1986). The hippocampus was immediately frozen in liquid nitrogen and stored at −80 °C until analyzed. Glucose (Glucose C2; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), insulin (Morinaga Ultra Sensitive Mouse/Rat Insulin ELISA Kit; Morinaga Institute of Biological Science, Yokohama, Japan), corticosterone (Corticosterone Enzyme Immunoassay Kit; Arbor Assays, Ann Arbor, MI, USA) and IGF-1 (Mouse/Rat IGF-1 Quantikine ELISA Kit; R&D Systems, Inc., Minneapolis, MN, USA) were measured using commercially available kits.

2.4. Y-maze test

Spatial working memory was assessed by the Y-maze test. The Y-maze apparatus (Muromachi Co. Ltd., Tokyo, Japan) comprised three grey plastic arms (each 41.5 cm long, 4 cm wide, with 10-cm high walls) separated by 120° and randomly labeled A, B, and C. The task was performed as previously described (Sarnyai et al., 2000).

2.5. Continuous subcutaneous administration of dexamethasone (DEX)

Mice (10–12 weeks old) were anesthetized with isoflurane and a 7-day micro-osmotic pump (Alzet Model 1007D, DURECT Corporation, Cupertino, CA, USA) was implanted subcutaneously between the shoulder blades. DEX is a synthetic glucocorticoid and have a similar long-lasting action to cortisol and corticosterone via glucocorticoid receptor (Mulatero et al., 1997). The pumps contained DEX sodium phosphate (10, 30 and 100 μg/day, FUJIFILM Wako Pure Chemical Corporation) dissolved in saline. Mice in the control group were given subcutaneous injections of an equal volume of saline. Seven days after implanting the micro-osmotic pumps, the mice were killed by an overdose of isoflurane for collection of brain region.

2.6. Intraperitoneal administration of the selective IGF-1 receptor tyrosine kinase inhibitor picropodophyllin (PPP)

PPP (20 mg/kg, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was dissolved in dimethyl sulfoxide (DMSO) and diluted with saline to a final concentration of 50 % DMSO (Menu et al., 2006). Mice in the control group were given intraperitoneal injections of an equal volume of vehicle (50 % DMSO in saline, 10 ml/kg body weight). The mice were killed by an overdose of isoflurane 1 h after the administration and the brain region was collected.

2.7. Intracerebroventricular injection of the CX3CR1 antagonist 18a

Intracerebroventricular injection was performed as described previously (Yamada-Goto et al., 2013). The highly selective antagonist for CX3CR1 18a (Axon Medchem, Groningen, Netherlands) with a Ki value of 3.9 nM, was dissolved in DMSO, and diluted with saline to a final concentration of 0.1 % DMSO (Karlström et al., 2013). The CX3CR1 antagonist 18a (50 ng/mouse) was intracerebroventricularly injected at 30 min before the Y-maze test. Mice in the control group were given intracerebroventricular injections of an equal volume of vehicle (0.1 % DMSO in saline, 2 μl/mouse).

2.8. Mouse primary neuron, astrocyte and microglia cultures

Mouse primary neuron and astrocyte cultures were performed as described previously (Katsuura et al., 1989; Yamada et al., 2009). According to a previous report (Han et al., 2013), primary microglia cell cultures were prepared from C57BL/6 J mouse brain on postnatal day 3. DEX (FUJIFILM Wako Pure Chemical Corporation), insulin (Thermo Fisher Scientific Inc., Waltham, MA, USA) and IGF-1 (PeproTech, Inc.,

Rocky Hill, NJ, USA) were used in this study. The purity of each cell culture was greater than 95 %.

2.9. Reverse transcription-polymerase chain reaction (RT-PCR)

The mRNA levels of fractalkine and CX3CR1 were measured by quantitative real-time RT-PCR as previously described (Yamada et al., 2009). All gene-specific mRNA expression values were normalized against the internal housekeeping gene 18S in the experiments involving the subcutaneous administration of DEX and the application of DEX in astrocyte cultures, or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in other experiments. Primers for GAPDH were as follows: [sense TGCACCACCAACTGCTTAGC, antisense GGATGCAGGGATGATGTTCTG], for 18S, [sense GTAACCCGTTGAACCCCATT, antisense CCATCCAATCGGTAGTAGCG], for fractalkine, [sense ACGAAATGCGAAATCATGTGC, antisense CTGTGTCGTCTCCAGGACAA], for CX3CR1, [sense CGTGAGACTGGGTGAGTGAC, antisense AAGGAGGTGGACATGGTGAG].

2.10. Western blotting analysis

Western blotting was performed as described previously (Yamada et al., 2011). The primary antibodies used in the present study were a rabbit polyclonal anti-CX3CL1 (fractalkine) antibody (ab25088, abcam, Cambridge, UK), a mouse monoclonal anti-CX3CR1 antibody (ab184678, abcam) and a mouse monoclonal anti-GAPDH antibody (sc-32233, Santa Cruz Biotechnology, Inc.). The secondary antibodies were an anti-rabbit IgG antibody conjugated to horseradish peroxidase (NA934, GE HealthCare UK Ltd., Buckinghamshire, UK) and an anti-mouse IgG antibody conjugated to horseradish peroxidase (NA931, GE HealthCare UK Ltd.).

2.11. Data analysis

Data are expressed as mean ± SEM. Statistical analysis of the data was performed by ANOVA followed by the Tukey-Kramer test. Statistical significance was defined as $P < 0.05$.

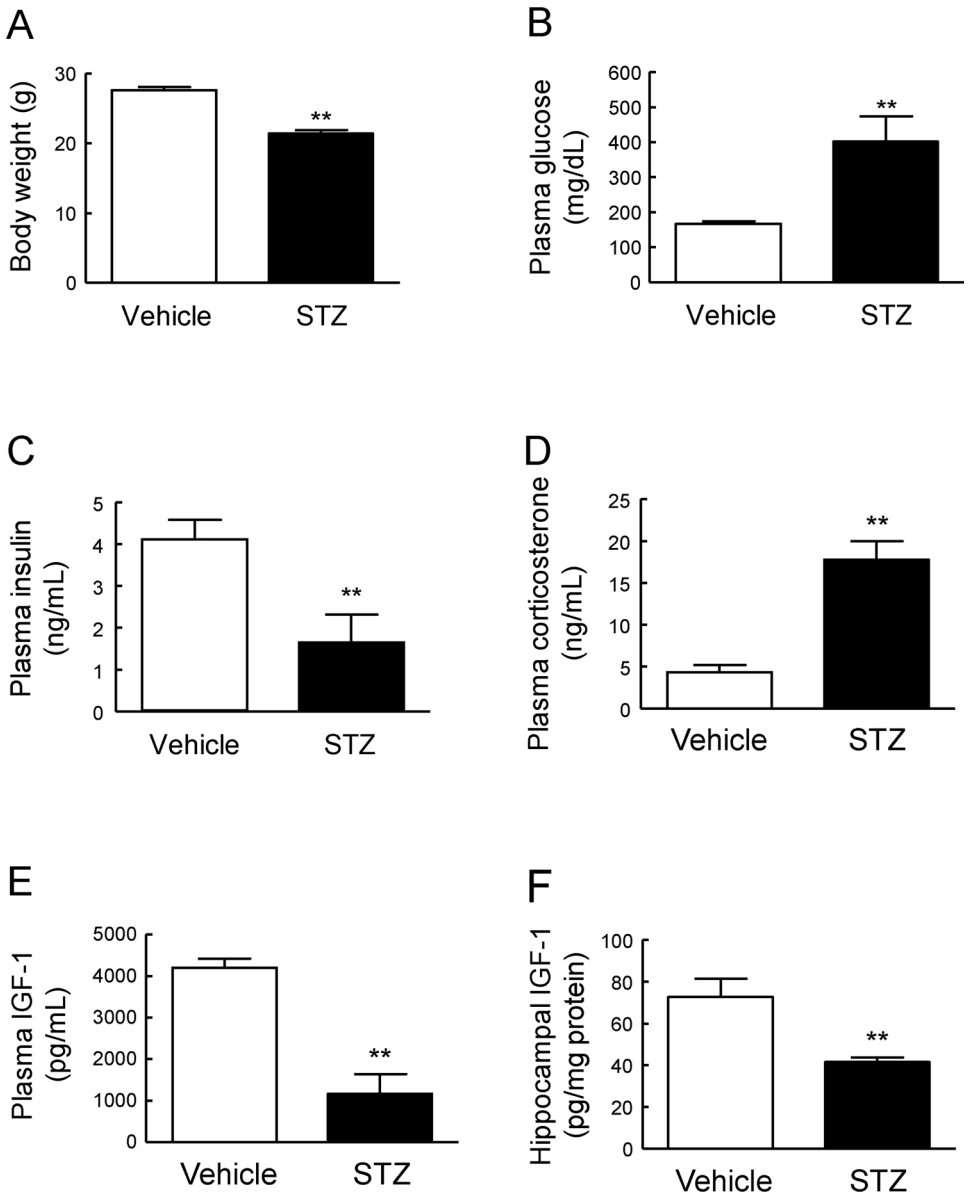

Fig. 1. Changes in body weight, plasma levels of glucose, insulin, corticosterone and IGF-1, and protein levels of IGF-1 in the hippocampus in STZ-treated mice. (A) Body weight, (B) Plasma glucose levels, (C) Plasma insulin levels, (D) Plasma corticosterone levels, (E) Plasma IGF-1 levels, (F) IGF-1 protein levels in the hippocampus. Results are expressed as mean ± SE for 9 to 17 mice. **p < 0.01 vs. vehicle.

3. Results

3.1. Changes in body weight, plasma levels of glucose, insulin, IGF-1 and corticosterone, and protein levels of IGF-1 in the hippocampus in STZ-treated mice

STZ-treated mice had a significantly lower body weight than vehicle-treated mice at two weeks after STZ injection (Fig. 1A). In STZ-treated mice, plasma glucose levels were markedly increased, while plasma insulin levels were significantly decreased compared with vehicle-treated mice (Fig. 1B and C). In addition, STZ treatment significantly increased plasma corticosterone levels to 414 % of those in vehicle-treated mice (Fig. 1D) and significantly decreased plasma IGF-1 levels to 28 % of those in vehicle-treated mice (Fig. 1E). The IGF-1 protein levels in the hippocampus of STZ-treated mice were significantly decreased to 56 % of those in vehicle-treated mice (Fig. 1F).

3.2. Changes in mRNA expression and protein levels of fractalkine and CX3CR1 in the hippocampus of STZ-treated mice

At two weeks after STZ injection, the mRNA expression and protein levels of fractalkine were significantly decreased in the hippocampus of STZ-treated mice to 73 % and 65 %, respectively, of those in vehicle-treated mice (Fig. 2A and B). Moreover, the mRNA expression and protein levels of CX3CR1 were significantly decreased in the hippocampus of STZ-treated mice to 70 % and 61 %, respectively, of those in vehicle-treated mice (Fig. 2C and D).

3.3. Cognitive ability in the Y-maze test

At two weeks after STZ injection, spontaneous alternation was significantly reduced in STZ-treated mice to 72 % of that in vehicle-treated mice (Fig. 3A). The number of entries into each arm was not different between groups (Fig. 3B).

To elucidate the role of fractalkine-CX3CR1 signaling in the learning and memory processes, we examined the effect of the CX3CR1 antagonist 18a on learning and memory in the Y-maze test in normal mice. Intracerebroventricular injection with 18a (50 ng/mouse) significantly reduced spontaneous alternation in mice to 78 % of that in vehicle-treated mice (Fig. 3C). The number of entries into each arm was not changed in 18a-treated mice (Fig. 3D)

3.4. Effects of the application of DEX, insulin and IGF-1 on mRNA expression of fractalkine in primary neuron and astrocyte cultures, and mRNA expression of CX3CR1 in primary microglia cultures

In primary neuron cultures, the application of DEX at concentrations of 10, 100 and 1000 nM for 24 h significantly deceased fractalkine mRNA expression (Fig. 4A). The application of insulin at a concentration of 1000 nM for 24 h did not change the fractalkine mRNA expression in primary neuron cultures (Fig. 4B). On the other hand, the application of IGF-1 at a concentration of 1000 nM for 24 h to primary neuron cultures significantly increased the fractalkine mRNA expression to 130 % of that in the control group (Fig. 4C).

In primary astrocyte cultures, the application of 10, 100 and 1000 nM DEX for 24 h significantly decreased fractalkine mRNA expression to 67 %, 48 % and 55 %, respectively, of that in the control group (Fig. 4D). Applications of insulin (1000 nM) and IGF-1 (1000 nM) for 24 h did not change the fractalkine mRNA expression in primary astrocyte cultures (Fig. 4E and F).

In primary microglia cultures, the application of 10, 100 and 1000 nM DEX for 24 h markedly decreased CX3CR1 mRNA expression to 77 %, 31 % and 31 %, respectively, of that in the control group (Fig. 4G). The application of insulin (1000 nM) for 24 h did not change CX3CR1 mRNA expression in primary microglia cultures (Fig. 4H). On the other hand, the application of 1000 nM IGF-1 for 24 h significantly

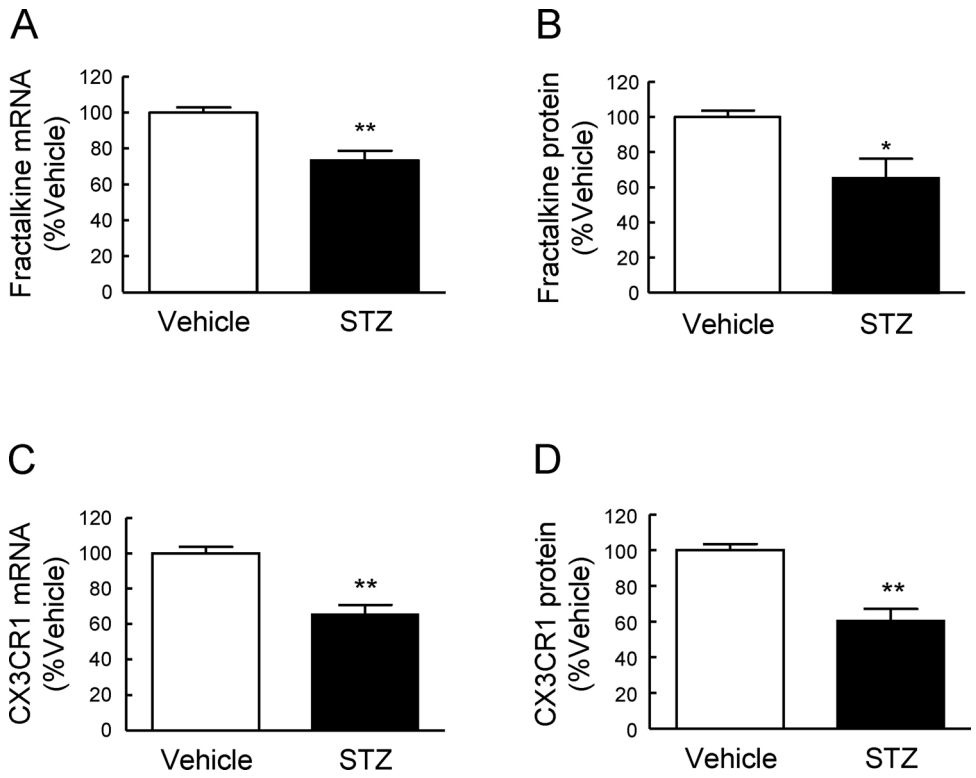

Fig. 2. Changes in the mRNA expression and protein levels of fractalkine and CX3CR1 in the hippocampus of STZ-treated mice. (A) Fractalkine mRNA expression, (B) Fractalkine protein levels, (C) CX3CR1 mRNA expression, (D) CX3CR1 protein levels. Results are expressed as mean ± SE for 5-16 mice. *$p < 0.05$, **$p < 0.01$ vs. vehicle.

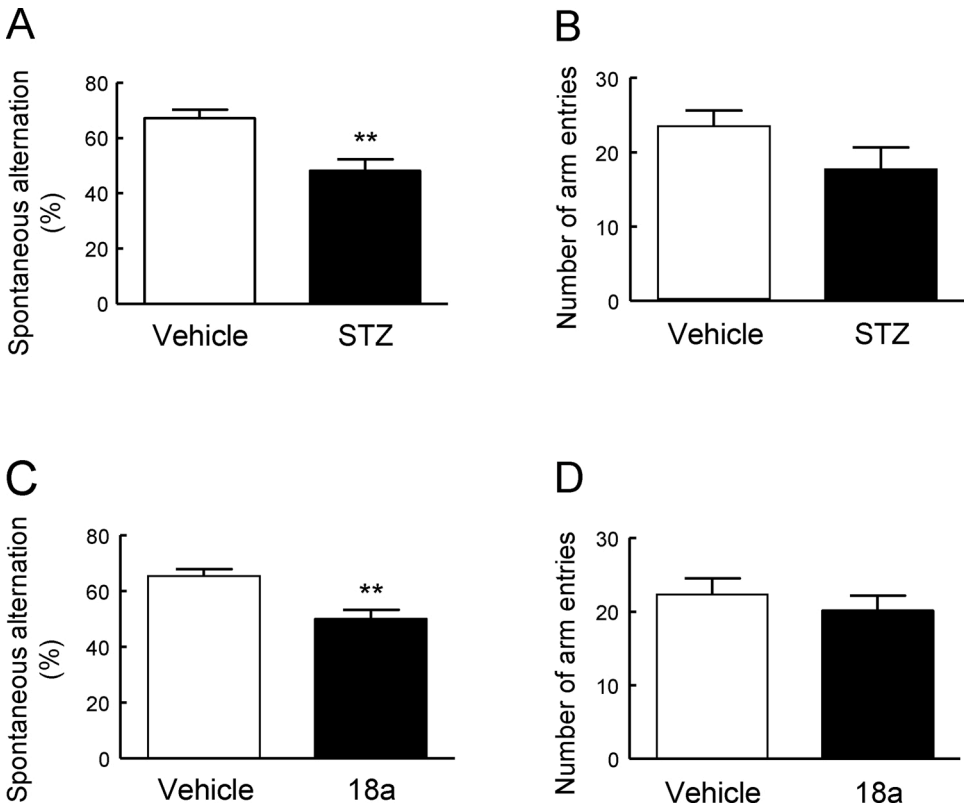

Fig. 3. Cognitive ability in the Y-maze test. STZ: (A) Spontaneous alternation (%) and (B) Number of arm entries. CX3CR1 antagonist 18a: (C) Spontaneous alternation (%) and (D) Number of arm entries. Results are expressed as mean ± SE for 9 to 11 mice. **p < 0.01 vs. vehicle.

increased the CX3CR1 mRNA expression in primary microglia cultures to 136 % of that in the control group (Fig. 4I).

3.5. Effects of the administration of DEX and selective IGF-1 receptor tyrosine kinase inhibitor PPP on mRNA expression of fractalkine and CX3CR1 in the mouse hippocampus

In the normal mice hippocampus, subcutaneous administration of DEX in doses of 30 and 100 μg/day for 7 days using a micro-osmotic pump significantly decreased fractalkine mRNA expression to 80 % and 73 %, respectively, of that in the saline-treated group, and significantly decreased CX3CR1 mRNA expression in a dose of 100 μg/day to 68 % of that in the saline-treated group (Fig. 5A and B).

Intraperitoneal injection of PPP (20 mg/kg body weight) in normal mice significantly decreased mRNA expression of fractalkine and CX3CR1 in the hippocampus to 77 % and 72 %, respectively, of that in vehicle-treated mice (Fig. 5C and D).

4. Discussion

In the present study, STZ-treated mice exhibited significant learning and memory impairment in the Y-maze test compared with vehicle-treated mice, and, moreover, a significant reduction in fractalkine and CX3CR1 mRNA levels in the hippocampus. Furthermore, intracerebroventricular injection of the CX3CR1 antagonist 18a in normal mice induced significant cognitive deficits in the Y-maze test. STZ-treated mice had high levels of plasma corticosterone, and low levels of plasma and hippocampal IGF-1 compared with vehicle-treated mice. Furthermore, DEX significantly decreased fractalkine mRNA expression in primary neuron and astrocyte cultures, and CX3CR1 mRNA expression in primary microglia cultures. On the other hand, IGF-1 significantly increased the fractalkine mRNA expression in primary neuron cultures and the CX3CR1 mRNA expression in primary microglia cultures. Moreover, subcutaneous administration of DEX significantly reduced the fractalkine and CX3CR1 mRNA expression in the hippocampus. Intraperitoneal administration of the IGF-1 receptor tyrosine kinase inhibitor PPP significantly decreased the fractalkine and CX3CR1 mRNA expression in the hippocampus. These findings suggest that the cognitive deficits exhibited by STZ-treated mice are, at least in part, due to impaired fractalkine-CX3CR1 signaling in the hippocampus induced by an increase in plasma corticosterone levels and a decrease in plasma and hippocampal IGF-1 levels.

In the hippocampus, fractalkine expression is predominantly restricted to glutamatergic pyramidal neurons in the CA1-CA3 and granule neurons in the dentate gyrus which are well-known to be potently involved in the processing of learning and memory (Nishiyori et al., 1998). Activation of CX3CR1 on microglia in the hippocampal CA1 region triggers the release of adenosine that in turn, via the activation of adenosine receptor type A2, increases the release of D-serine as a coagonist for the N-methyl-D-aspartate (NMDA) glutamate receptor subtype from glia, thereby potentiating NMDA function (Scianni et al., 2013). LTP, defined as an activity-dependent, prolonged enhancement of synaptic strength, in the hippocampal CA1 region is predominantly regulated by glutamate receptors, such as NMDA and α-amino-3--hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and is considered to be a form of synaptic plasticity for the cellular mechanisms of learning and memory in the hippocampus (Neves et al., 2008). These findings suggest that fractalkine-CX3CR1 signaling plays an important role in learning and memory processes in association with enhanced LTP (Rogers et al., 2011; Sheridan et al., 2014). In the present study, administration of a CX3CR1 antagonist significantly impaired learning and memory in the Y maze test. Supporting our result, Rogers et al. reported that male CX3CR1-deficient mice showed cognitive impairment in fear conditioning and Morris water maze test (Rogers et al., 2011). In Morris water maze test, CX3CR1-deficient mice had a significant decrease in the number of target platform crossing during the

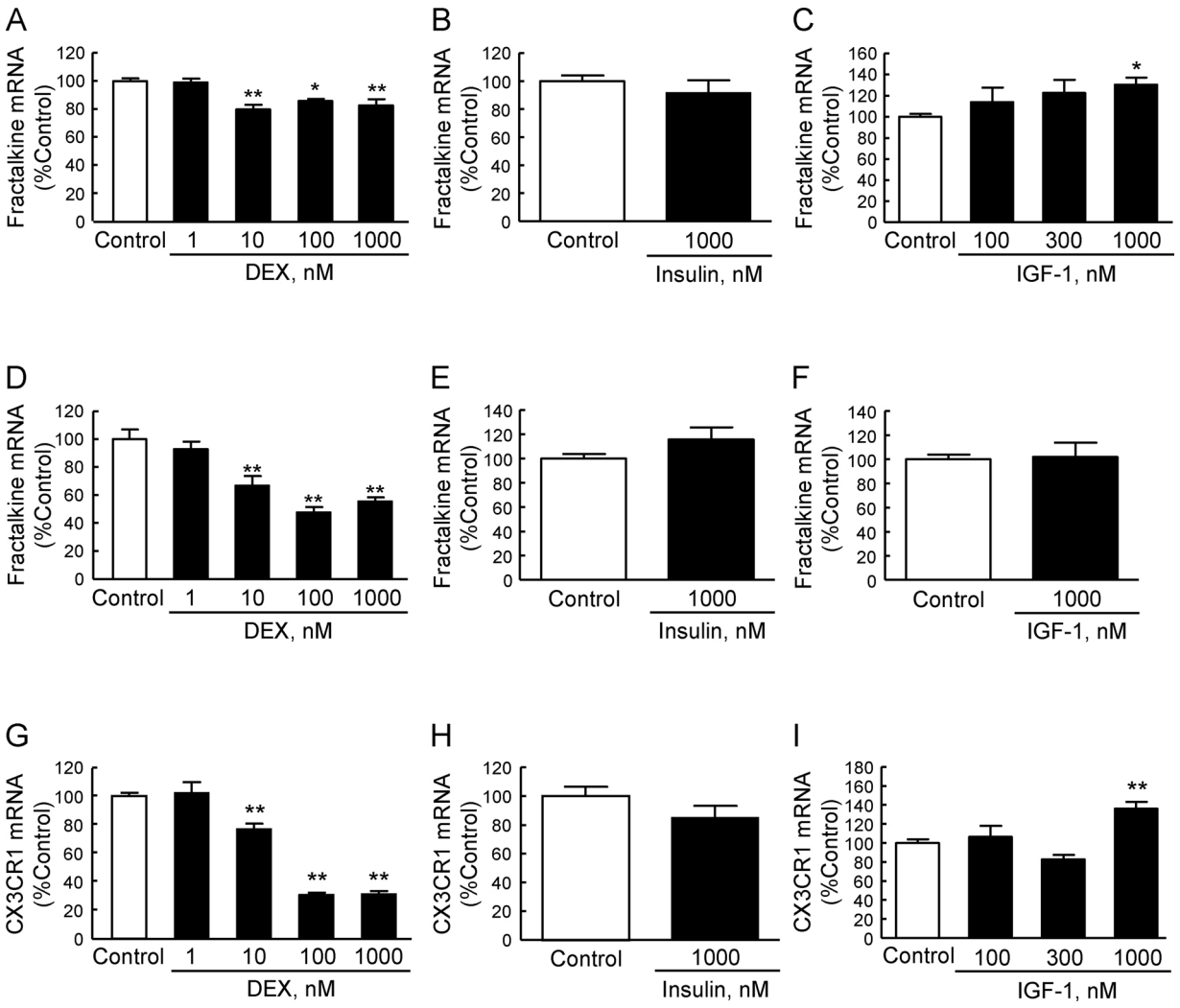

Fig. 4. Effects of the application of DEX, insulin and IGF-1 on mRNA expression of fractalkine in the primary neuron and astrocyte cultures, and mRNA expression of CX3CR1 in the primary microglia cultures. Fractalkine mRNA expression in the primary neuron cultures: (A) DEX, (B) insulin, (C) IGF-1. Fractalkine mRNA expression in the primary astrocyte cultures: (D) DEX, (E) insulin, (F) IGF-1. CX3CR1 mRNA expression in the primary microglia cultures: (G) DEX, (H) insulin, (I) IGF-1. Results are expressed as mean ± SE for 6 to 20 samples. *$p < 0.05$, **$p < 0.01$ vs. control.

probe trial (Day 11) compared to wild-type mice, indicating cognitive impairment in CX3CR1-deficient mice (Rogers et al., 2011). On the other hand, Maggi et al. reported that female CX3CR1-deficient mice learned the water maze task faster than wild-type mice because CX3CR1-deficient mice significantly spent in the acquisition quadrant longer than wild-type mice during probe trial (Day 4) (Maggi et al., 2011). However, on probe trial (Day 6), spent time in the acquisition quadrant was not different between two groups (Maggi et al., 2011). We consider that the difference in these results may be due, in part, to the differences in experimental schedule and gender. Moreover, Rogers et al. reported that male CX3CR1-deficient mice showed significantly reduced hippocampal-dependent LTP compared to wild-type mice (Rogers et al., 2011). However, Maggi et al. reported that CX3CR1-deficient mice showed an increase in AMPA receptor-mediated LTP but not NMDA receptor-mediated LTP (Maggi et al., 2011). On the other hand, another report of Maggi showed that fractalkine enhanced hippocampal NMDA receptor-dependent LTP in mice (Scianni et al., 2013). In this context, the same laboratory demonstrated different results which might be due to the different experimental methods. As compared with these two studies on CX3CR1-deficient mice, our experiment was performed using CX3CR1 antagonist. The big difference between Rogers/Maggi studies and ours is permanent intervention and acute intervention, respectively. Taken together, these findings provide compelling evidence that neuron-microglia interactions, especially those underpinned by fractalkine-CX3CR1 signaling, play several crucial roles in modulating hippocampal-dependent learning and memory by maintaining proper homeostasis of synaptic transmission in the brain.

DM is a common serious metabolic disorder characterized by hyperglycemia resulting from defective insulin activity. Diabetes may lead to secondary complications in several organ systems, including the brain (Biessels et al., 1994, 2008). A growing number of studies on brain function have revealed moderate impairment of cognitive function is recognized as a complication of type 1 DM (Neves et al., 2008). Initially, deficient insulin actions may be considered to contribute to the cognitive impairment observed in DM because insulin positively regulates cognitive processing, and impaired insulin activity in the brain leads to impaired neuronal function and synaptogenesis (Kleinridders et al., 2014). The multifactorial pathogenesis of brain dysfunction, such as cognitive impairment in DM, however, is not yet completely understood. STZ-treated mice, a widely used model of type 1 DM with hypoinsulinemia, hyperglycemia and reduced body weight, exhibit cognitive deficits in association with impaired LTP in the hippocampal CA1 region (Biessels et al., 1996; Kamal et al., 1999, 2000; Molteni et al., 2002; Wu et al., 2004). At least part of the learning and synaptic plasticity deficits in STZ-treated rats may be a direct consequence of disturbances at the level of the NMDA and AMPA receptor complexes in the hippocampus

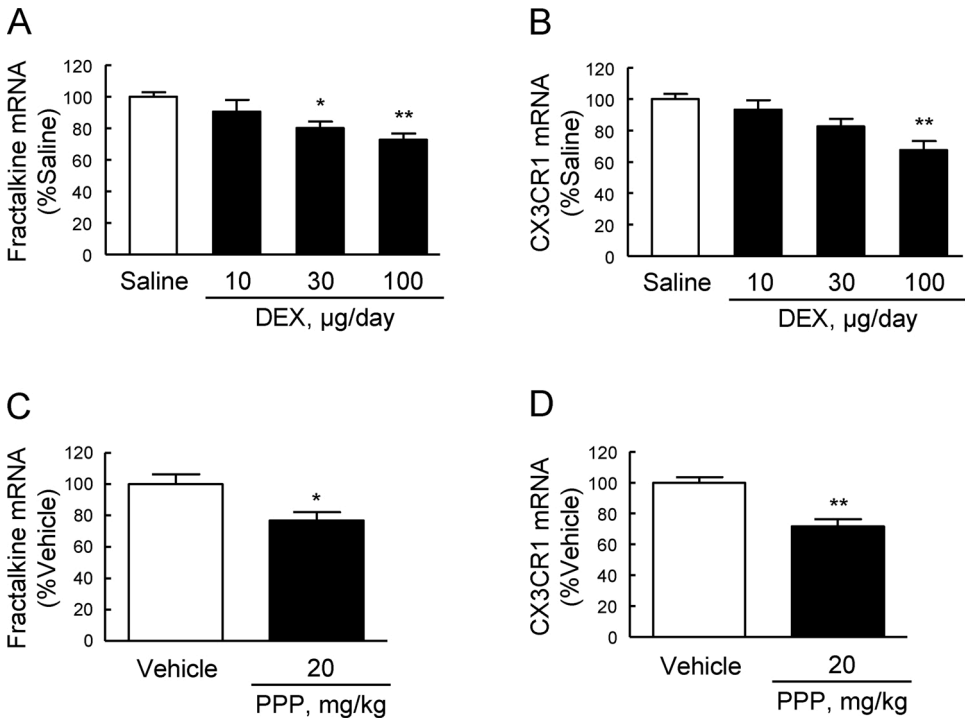

Fig. 5. Effects of the administration of DEX and IGF-1 receptor tyrosine kinase inhibitor PPP on mRNA expression of fractalkine and CX3CR1 in mouse hippocampus. DEX: (A) Fractalkine mRNA expression, (B) CX3CR1 mRNA expression. PPP: (C) Fractalkine mRNA expression, (D) CX3CR1 mRNA expression. Results are expressed as mean ± SE for 6 to 9 samples. *$p < 0.05$, **$p < 0.01$ vs. saline or vehicle.

(Sasaki-Hamada et al., 2012). Concretely, the NMDA receptor NR2B subunits and the AMPA receptor GluR1 subunits are significantly decreased in the hippocampus of STZ-treated animals (Gardoni et al., 2002; Viswaprakash et al., 2015). Based on previous findings suggesting the participation of fractalkine-CX3CR1 signaling in learning and memory processes, we addressed whether fractalkine-CX3CR1 signaling in the brain is involved in cognitive deficits in STZ-treated mice. The present study demonstrated that STZ-treated mice exhibited significant decrease in fractalkine-CX3CR1 signaling in the hippocampus accompanied by cognitive deficits. On the basis of these findings, the impaired learning and memory in STZ-treated mice is, in part, attributed to reduced fractalkine-CX3CR1 signaling in the hippocampus.

To elucidate the mechanisms underlying the decreased expression of fractalkine and CX3CR1 in the hippocampus of STZ-treated mice, we examined the effects of factors observed to be significantly changed in the plasma and hippocampus of STZ-treated mice in the present study, such as corticosterone, insulin and IGF-1, on the mRNA expression of fractalkine and CX3CR1. Type 1 DM is associated with significantly higher plasma cortisol and adrenocorticotrophic hormone levels compared with normal controls (Chan et al., 2002). DEX which is a synthetic glucocorticoid receptor agonist significantly suppresses increases in the protein levels and mRNA expression of fractalkine induced by tumor necrosis factor-α and interferon-γ in a human lung epithelial adenocarcinoma cell line (Bhavsar et al., 2008). Moreover, application of DEX reduces CX3CR1 mRNA expression in human peripheral blood mononuclear cells (Pachot et al., 2008). Consistent with these findings, the present study showed that plasma corticosterone levels were significantly increased in STZ-treated mice. Moreover, cell culture studies showed that DEX significantly reduced fractalkine-CX3CR1 signaling. In the present study, subcutaneous administration of DEX in normal mice significantly reduced the mRNA expression of fractalkine and CX3CR1 in the hippocampus. These findings suggest that high plasma corticosterone levels in STZ-treated mice contribute to reduce fractalkine and CX3CR1 expression in the brain. Recent observations revealed that circulating and brain IGF-1, which acts as trophic factor, modulates brain activities such as neuroprotection, neurogenesis and neuronal excitability (Jones and Clemmons, 1995). Serum IGF-1-deficient mice exhibit both cognitive decline and impaired hippocampal LTP (Trejo et al., 2007). Serum and brain IGF-1 levels are reduced in STZ-treated rats (Olchovsky et al., 1990), and systemic administration of IGF-1 prevents cognitive impairment in STZ-treated rats (Lupien et al., 2003). These findings provide substantial evidence that diabetic patients may have diminished brain IGF-1 signaling as well as insulin signaling. The present study demonstrated that plasma and hippocampal IGF-1 levels were significantly decreased in STZ-treated mice. Moreover, IGF-1 significantly increased mRNA expression of fractalkine in primary neuron cultures and of CX3CR1 in primary microglia cultures. In contrast, intraperitoneal administration of the IGF-1 receptor tyrosine kinase inhibitor PPP significantly decreased mRNA expression of fractalkine and CX3CR1 in the hippocampus. These findings indicate that impaired fractalkine-CX3CR1 signaling in the hippocampus in STZ-treated mice is, in part, attributed to a decrease in plasma and hippocampal IGF-1 levels. However, it is unclear mechanism how corticosterone and IGF-1 change the expression of fractalkine and CX3CR1.

Our findings revealed that STZ treatment induces a significant decrease in fractalkine-CX3CR1 signaling in the hippocampus, which in turn, may result in cognitive impairment. Moreover, reduced fractalkine-CX3CR1 signaling seems to be induced by an increase in plasma corticosterone levels and a decrease in plasma and hippocampal IGF-1 levels. Thus, interactions between neurons and microglia regulated by fractalkine and CX3CR1 appear to be involved in the cognitive deficiency associated with type1 DM.

Author contributions

N.K. and G.K. performed experiments, contributed to discussions, and wrote the manuscript. E.N. performed experiments. NY.G., A.I. and A.A. contributed to discussions, and reviewed and edited the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Namiko Kawamura: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Goro Katsuura:** Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision. **Nobuko Yamada-Goto:** Investigation. **Ela Novianti:** Investigation. **Akio Inui:** Resources, Writing - review & editing, Funding acquisition. **Akihiro Asakawa:** Resources, Writing - review & editing.

Acknowledgments

We wish to thank Joint Research Laboratory, Kagoshima University Graduate School of Medical and Dental Sciences, for the use of their facilities.

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology in Japan (16H06404 to AI).

References

Bhavsar, P.K., Sukkar, M.B., Khorasani, N., Lee, K.Y., Chung, K.F., 2008. Glucocorticoid suppression of CX3CL1 (fractalkine) by reduced gene promoter recruitment of NF-κB. FASEB J. 22, 1807–1816.

Biessels, G.J., Kappelle, A.C., Bravenboer, B., Erkelens, D.W., Gispen, W.H., 1994. Cerebral function in diabetes mellitus. Diabetologia 37, 643–650.

Biessels, G.J., Kamal, A., Ramakers, G.M., Urban, I.J., Spruijt, B.M., Erkelens, D.W., Gispen, W.H., 1996. Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. Diabetes 45, 1259–1266.

Biessels, G.J., Deary, I.J., Ryan, C.M., 2008. Cognition and diabetes: a lifespan perspective. Lancet Neurol. 7, 184–190.

Chan, O., Inouye, K., Vranic, M., Matthews, S.G., 2002. Hyperactivation of the hypothalamo-pituitary-adrenocortical axis in streptozotocin-diabetes is associated with reduced stress responsiveness and decreased pituitary and adrenal sensitivity. Endocrinology 143, 1761–1768.

Gardoni, F., Kamal, A., Bellone, C., Biessels, G.J., Ramakers, G.M., Cattabeni, F., Gispent, W.H., Di Luca, M., 2002. Effects of streptozotocin-diabetes on the hippocampal NMDA receptor complex in rats. J. Neurochem. 80, 438–447.

Goazigo, A.R.-L., Van Steenwinckel, J., Rosténe, W., Parsadaniantz, S.M., 2013. Current status of chemokines in the adult CNS. Prog. Neurobiol. 104, 67–92.

Han, L., Yin, K., Zhang, S., Wu, Z., Wang, C., Zhang, Q., Pan, J., Chen, B., Li, J., Tan, R., Xu, Y., 2013. Dalesconols B inhibits lipopolysaccharide induced inflammation and suppresses NF-κB and p38/JNK activation in microglial cells. Neurochem. Int. 62, 913–921.

Imai, T., Hieshima, K., Haskell, C., Baba, M., Nagira, M., Nishimura, M., Kakizaki, M., Takagi, S., Nomiyama, H., Schall, T.J., Yoshie, O., 1997. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. Cell 91, 521–530.

Jones, J.I., Clemmons, D.R., 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev. 16, 3–34.

Kamal, A., Biessels, G.J., Urban, I.J., Gispen, W.H., 1999. Hippocampal synaptic plasticity in streptozotocin-diabetic rats: impairment of long-term potentiation and facilitation of long-term depression. Neuroscience 90, 737–745.

Kamal, A., Biessels, G.J., Duis, S.E., Gispen, W.H., 2000. Learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: interaction of diabetes and ageing. Diabetologia 43, 500–506.

Karlström, S., Nordvall, G., Sohn, D., Hettman, A., Turek, D., Åhlin, K., Kers, A., Claesson, M., Slivo, C., Lo-Alfredsson, Y., Petersson, C., Bessidskaia, G., Svensson, P. H., Rein, T., Jerning, E., Malmberg, Å, Ahlgen, C., Ray, C., Vares, L., Ivanov, V., Johansson, R., 2013. Substituted 7-amino-5-thio-thiazolo[4,5-d]pyrimidines as potent and selective antagonists of the fractalkine receptor (CX3CR1). J. Med. Chem. 56, 3177–3190.

Katsuura, G., Gottschall, P.E., Dahl, R.R., Arimura, A., 1989. Interleukin-1 beta increases prostaglandin E2 in rat astrocyte cultures: modulatory effect of neuropeptides. Endocrinology 124, 3125–3127.

Kleinridders, A., Ferris, H.A., Cai, W., Kahn, C.R., 2014. Insulin action in brain regulates systemic metabolism and brain function. Diabetes 63, 2232–2243.

Lupien, S.B., Bluhm, E.J., Ishii, D.N., 2003. Systemic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. J. Neurosci. Res. 74, 512–523.

Maciejewski-Lenoir, D., Chen, S., Feng, L., Maki, R., Bacon, K.B., 1999. Characterization of fractalkine in rat brain cells: migratory and activation signals for CX3CR-1-expressing microglia. J. Immunol. 163, 1628–1635.

Maggi, L., Scianni, M., Branchi, I., D'Andrea, I., Lauro, C., Limatola, C., 2011. CX3CR1 deficiency alters hippocampal-dependent plasticity phenomena blunting the effects of enriched environment. Front. Cell. Neurosci. 5, 22.

McCall, A.L., 1992. The impact of diabetes on the CNS. Diabetes 41, 557–570.

Menu, E., Jernberg-Wiklund, H., Stromberg, T., De Raeve, H., Girnita, L., Larsson, O., Axelson, M., Asosingh, K., Nilsson, K., Van Camp, B., Vanderkerken, K., 2006. Inhibiting the IGF-1 receptor tyrosine kinase with the cyclolignan PPP: an in vitro and in vivo study in the 5T33MM mouse model. Blood 107, 655–660.

Molteni, R., Barnard, R.J., Ying, Z., Roberts, C.K., Gómez-Pinilla, F., 2002. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 112, 803–814.

Mulatero, P., Panarelli, M., Schiavone, D., Rossi, A., Mengozzi, G., Kenyon, C.J., Chiandussi, L., Veglio, F., 1997. Impaired cortisol binding to glucocorticoid receptors in hypertensive patients. Hypertension 30, 1274–1278.

Nakao, K., Katsuura, G., Morii, N., Itoh, H., Shiono, S., Yamada, T., Sugawara, A., Sakamoto, M., Saito, Y., Eigyo, M., Matsushita, A., Imura, H., 1986. Inhibitory effect of centrally administered atrial natriuretic polypeptide on the brain dopaminergic system in rats. Eur. J. Pharmacol. 131, 171–177.

Neves, G., Cooke, S.F., Bliss, T.V., 2008. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nat. Rev. Neurosci. 9, 65–75.

Nishiyori, A., Minami, M., Ohtani, Y., Takami, S., Yamamoto, J., Kawaguchi, N., Nume, T., Akaike, A., Satoh, M., 1998. Localization of fractalkine and CX3CR1 mRNAs in rat brain: does fractalkine play a role in signaling from neuron to microglia? FEBS Lett. 429, 167–172.

Olchovsky, D., Bruno, J.F., Wood, T.L., Gelato, M.C., Leidy Jr, J.W., Gilbert Jr, J.M., Berelowitz, M., 1990. Altered pituitary growth hormone (GH) regulation in streptozotocin-diabetic rats: a combined defect of hypothalamic somatostatin and GH-releasing factor. Endocrinology 126, 53–61.

Pachot, A., Cazalis, M.A., Venet, F., Turrel, F., Faudot, C., Voirin, N., Diasparra, J., Bourgoin, N., Poitevin, F., Mougin, B., Lepape, A., Monneret, G., 2008. Decreased expression of the fractalkine receptor CX3CR1 on circulating monocytes as new feature of sepsis-induced immunosuppression. J. Immunol. 180, 6421–6429.

Rogers, J.T., Morganti, J.M., Bachstetter, A.D., Hudson, C.E., Peters, M.M., Grimmig, B. A., Weeber, E.J., Bickford, P.C., Gemma, C., 2011. CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J. Neurosci. 31, 16241–16250.

Sarnyai, Z., Sibille, E.L., Pavlides, C., Fenster, R.J., McEwen, B.S., Tóth, M., 2000. Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin1A receptors. Proc. Natl. Acad. Sci. U.S.A. 97, 14731–14736.

Sasaki-Hamada, S., Sacai, H., Oka, J.I., 2012. Diabetes onset influences hippocampal synaptic plasticity in streptozotocin-treated rats. Neuroscience 227, 293–304.

Scianni, M., Antonilli, L., Chece, G., Cristalli, G., Di Castro, M.A., Limatola, C., Maggi, L., 2013. Fractalkine (CX3CL1) enhances hippocampal N-methyl-D-aspartate receptor (NMDAR) function via D-serine and adenosine receptor type A2 (A2AR) activity. J. Neuroinflamm. 10, 108.

Sheridan, G.K., Wdowicz, A., Pickering, M., Watters, O., Halley, P., O'Sullivan, N.C., Mooney, C., O'Connell, D.J., O'Connor, J.J., Murphy, K.J., 2014. CX_3CL1 is up-regulated in the rat hippocampus during memory-associated synaptic plasticity. Front. Cell. Neurosci. 8, 233.

Trejo, J.L., Piriz, J., Llorens-Martin, M.V., Fernandez, A.M., Bolós, M., LeRoith, D., Nuñez, A., Torres-Aleman, I., 2007. Central actions of liver-derived insulin-like growth factor I underlying its pro-cognitive effects. Mol. Psychiatry 12, 1118–1128.

Viswaprakash, N., Vaithianathan, T., Viswaprakash, A., Judd, R., Parameshwaran, K., Suppiramaniam, V., 2015. Insulin treatment restores glutamate (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor function in the hippocampus of diabetic rats. J. Neurosci. Res. 93, 1442–1450.

Wu, A., Ying, Z., Gomez-Pinilla, F., 2004. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. Eur. J. Neurosci. 19, 1699–1707.

Yamada, N., Katsuura, G., Tatsuno, I., Kawahara, S., Ebihara, K., Saito, Y., Nakao, K., 2009. Orexins increase mRNA expressions of neurotrophin-3 in rat primary cortical neuron cultures. Neurosci. Lett. 450, 132–135.

Yamada, N., Katsuura, G., Ochi, Y., Ebihara, K., Kusakabe, T., Hosoda, K., Nakao, K., 2011. Impaired CNS leptin action is implicated in depression associated with obesity. Endocrinology 152, 2634–2643.

Yamada-Goto, N., Katsuura, G., Ochi, Y., Ebihara, K., Kusakabe, T., Hosoda, K., Nakao, K., 2012. Impairment of fear-conditioning responses and changes of brain neurotrophic factors in diet-induced obese mice. J. Neuroendocrinol. 24, 1120–1125.

Yamada-Goto, N., Katsuura, G., Ebihara, K., Inuzuka, M., Ochi, Y., Yamashita, Y., Kusakabe, T., Yasoda, A., Satoh-Asahara, N., Ariyasu, H., Hosoda, K., Nakao, K., 2013. Intracerebroventricular administration of C-type natriuretic peptide suppresses food intake via activation of the melanocortin system in mice. Diabetes 62, 1500–1504.

Yoshida, H., Imaizumi, T., Fujimoto, K., Matsuo, N., Kimura, K., Cui, X.-F., Matsumiya, T., Tanji, K., Shibata, T., Tama, W., Kumagai, M., Satoh, K., 2001. Synergistic stimulation, by tumor necrosis factor-α and interferon-γ, of fractalkine expression in human astrocytes. Neurosci. Lett. 303, 132–136.