ELSEVIER

European Journal of Pharmacology 363 (1998) 131–133

ejp

Short communication

A hyperalgesic effect of intracerebroventricular cytokine-induced neutrophil chemoattractant-1 in the rat paw pressure test

Junki Yamamoto, Atsushi Nishiyori, Shinya Takami, Yoshikazu Ohtani, Masabumi Minami, Masamichi Satoh *

Department of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, 606-8501, Japan

Received 9 July 1998; revised 27 October 1998; accepted 30 October 1998

Abstract

Cytokine-induced neutrophil chemoattractant-1 (CINC-1) is a member of the chemokine superfamily. The effect of intracerebroventricular (i.c.v) injection of CINC-1 on the mechanical nociceptive threshold in the rat was examined using the paw-pressure test. An i.c.v. injection of CINC-1 at doses of 1 and 10 pg/rat tended to decrease the nociceptive threshold for mechanical stimuli at 15 min after the injection, and significantly lowered the threshold at 30 min. The threshold stayed at these lowered level over 180 min after the injection. Lower (100 fg/rat) and higher (30 and 100 pg/rat, and 1 and 10 ng/rat) doses of CINC-1 had no effect on the mechanical nociceptive threshold. The present results suggest that CINC-1 facilitates mechanical nociception in the central nervous system (CNS).

Keywords: CINC-1 (Cytokine-induced neutrophil chemoattractant-1); Chemokine; Brain; Nociception; Mechanical stimuli

1. Introduction

Cytokine-induced neutrophil chemoattractant-1 (CINC-1), which is the counterpart of human growth-related oncogene product (GRO) (Watanabe et al., 1989a; Zagorski and Delarco, 1993), is an 8-kDa polypeptide isolated and purified from conditioned medium of the rat kidney epithelioid cell line, NRK-52E, stimulated with lipopolysaccharide, tumor necrosis factor-α (TNF-α) and interleukin-1β (Watanabe et al., 1989b). It is a potent chemotactic factor for rat neutrophils in vitro and in vivo (Watanabe et al., 1991) and belongs to the chemokine superfamily. To date, chemokines can be divided into four subfamilies, based on the motives of two cystein residues: CXC, CC, C, CX_3C chemokines, and CINC-1 is a member of the CXC chemokines which include interleukin-8.

It has been reported that intra-plantar (i.pl.) injection of interleukin-8 evokes a hyperalgesia in the rat paw pressure test which is attenuated by propranolol, atenolol, SCH23390 (*R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol) or guanethidine (Cunha et al., 1991). In our laboratory, it has been demonstrated that intracerebroventricular (i.c.v.) interleukin-1β produces biphasic effects on mechanical nociception in the rat paw pressure test (Yabuuchi et al., 1996). Chemokines and cytokines are remarkably responsive to multiple inflammations, and play an important role in the regulation of the immune response (Petrek et al., 1995). However, these findings suggest that chemokines and cytokines not only contribute to the immune system but also play a role as neuromodulator in the brain. Thus, it was of interest to examine the participation of CXC chemokines in nociception in the CNS. We are now investigating the expressions of chemokine receptors mRNAs, including CXC chemokine receptor-2 (CXCR2), which is the receptor for CINC-1, using molecular biological methods. In the present behavioral study, we investigated the effects of i.c.v. administration of CINC-1 on the mechanical nociceptive threshold in the rat, using the paw-pressure test done as a blind study.

2. Materials and methods

2.1. Animals and implantation of guide cannula

All experiments using male Sprague–Dawley rats (210–260 g, 6–7 weeks old) followed the ethical guide-

* Corresponding author. Tel.: +81-75-753-4526; Fax: +81-75-753-4586; E-mail: msatoh@pharm.kyoto-u.ac.jp

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00801-2

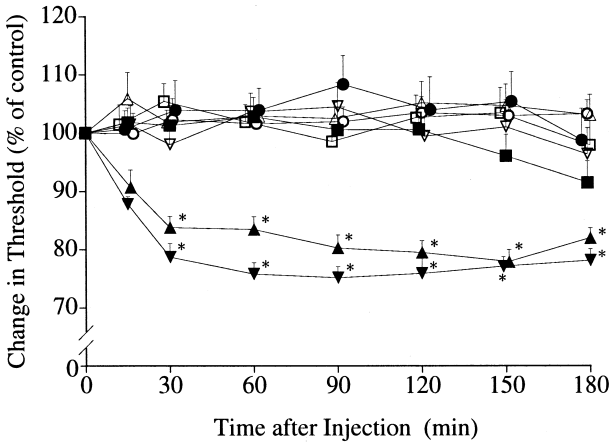

Fig. 1. Time courses of the effects of i.c.v. injection of CINC-1 on the mechanical nociceptive threshold. The nociceptive threshold at each time point is presented as mean percentage of the control ± S.E.M.. ○, vehicle ($n = 10$); □, CINC-1 100 fg/rat ($n = 10$); ▲, CINC-1 1 pg/rat ($n = 9$); ▼, CINC-1 10 pg/rat ($n = 10$); ■, CINC-1 30 pg/rat ($n = 9$); ●, CINC-1 100 pg/rat ($n = 9$); △, CINC-1 1 ng/rat ($n = 10$); ▽, CINC-1 10 ng/rat ($n = 10$). * $P < 0.05$ compared with the vehicle-injected group (Bonferroni's post-hoc test).

lines for investigations of experimental pain in conscious animals (Zimmermann, 1983). The animals were kept at a constant ambient temperature (24 ± 1°C) under a 12-h light and dark cycle with free access to food and water.

Under pentobarbital anesthesia (50 mg/kg i.p.), a stainless-steel guide cannula (o.d. 0.7 mm) was stereotaxically (P 0.8, L 1.5, H 2.0) implanted according to the atlas of Paxinos and Watson (1986). After surgery, the animals were returned to their individual cages.

2.2. Materials and drug administration

CINC-1 was purchased from the Peptide Institute (Minoh, Japan). All other chemicals were purchased from Nacalai Tesque (Kyoto, Japan).

CINC-1 was dissolved in phosphate-buffered saline (PBS, pH 7.6) containing 0.1% bovine serum albumin and was administered via the injection cannula which reached the lateral ventricle (P 0.8, L 1.5, H 4.0) when attached to the guide cannula. The drug was administered intracerebroventricularly in a volume of 5 μl at a constant rate of 10 μl/min. The doses of CINC-1 injected were not revealed to the experimenter until the nociceptive threshold had been measured and all data were analyzed.

2.3. Measuring the nocicepitve threshold

The nociceptive threshold of the hind paw for mechanical stimulation was measured using an analgesimeter (Ugo Basile, Milan, Italy) with a cone-shaped piston at a loading rate of 32 g/s, and the pressure eliciting paw withdrawal was determined. After 5 days of recovery after implantation of the guide cannula, the rats were habituated to the procedure for measuring the nociceptive threshold three times per day. After 2 days of habituation, the nociceptive threshold of each animal was measured following two additional habituation procedures, and the value for the threshold was taken as a control. The nociceptive thresholds were measured at 15, 30, 60, 90, 120, 150 and 180 min after the administration of CINC-1.

2.4. Statistical analysis

The nociceptive threshold at each time point is presented as the mean of the percentage of the control ± S.E.M. The areas under the curve (AUC) values were obtained by calculating the area between the zero line and the curve for the time course of the nociceptive threshold from 0 to 180 min after the administration of CINC-1. Differences were compared using Bonferroni's post-hoc test. $P < 0.05$ was considered significant.

3. Results

We examined the effect of i.c.v. injection of CINC-1 on the nociceptive threshold for paw-pressure stimulation (Fig. 1). The i.c.v. injection of vehicle did not alter the nociceptive threshold. At doses of 1 and 10 pg/rat, the threshold tended to decrease at 15 min, and was significantly lowered at 30 min, after the injection when compared with that of the vehicle-injected group. The nociceptive thresholds were decreased to 77.9% (150 min) and 75.2% (90 min) of the control level, respectively, and remained at these levels 180 min after drug administration. Lower (100 fg/rat) and higher (30 and 100 pg/rat, and 1 and 10 ng/rat) doses of CINC-1 had no effect on the nociceptive threshold.

The AUC values of the groups injected with 1 and 10 pg/rat of CINC-1 were significantly smaller than that of

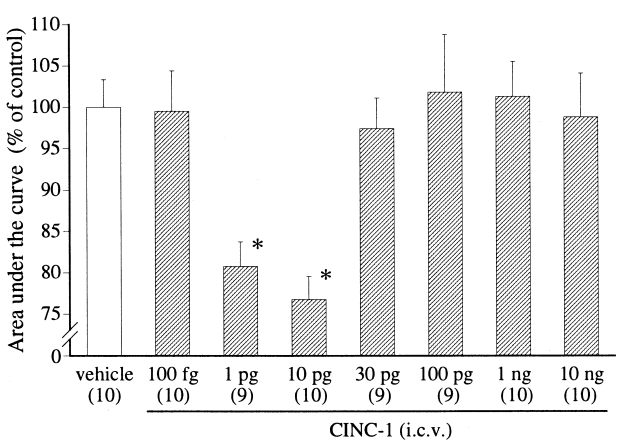

Fig. 2. The magnitudes of the effects of i.c.v. injection of CINC-1 on the mechanical nociceptive threshold are presented as the AUC values. Numbers of animals used are given in parentheses. * $P < 0.05$ compared with the vehicle-injected group (Bonferroni's post-hoc test).

the control group (Fig. 2). However, there was no significant difference in AUC values between the other groups and the control group.

4. Discussion

In this study, we demonstrated that CINC-1 administered intracerebroventricularly decreased the nociceptive threshold for mechanical stimuli. This hyperalgesic effect was caused only by 1 and 10 pg/rat of CINC-1, and the other doses of CINC-1 did not affect the nociceptive threshold. We also tested macrophage inflammatory protein-1α (MIP-1α) (1 pg/rat–10 ng/rat) which is a member of the CC chemokines on the mechanical nociceptive threshold, but it had no effects (data not shown). These results suggest that the CXC chemokines, but not CC chemokines, could be involved in the modulation of mechanical nociceptive transmission in the CNS.

Human interleukin-8 cDNA was cloned from blood mononuclear cells and its peptide was purified (Yoshimura et al., 1987); interleukin-8 belongs to CXC chemokine family, as well as CINC-1. Cunha et al. (1991) have reported that interleukin-8 injected into the hind paw (i.pl.) evokes a dose-dependent hyperalgesia in response to the mechanical stimuli, though its possible central effect on nociception was not investigated.

To date, several studies have shown that the chemokines mediated several central effects. At first, it was reported that micro-injection of MIP-1 into the anterior hypothalamic preoptic area caused a fever which was not blocked by indomethacin (Miñano et al., 1991). Thereafter, Zawada et al. (1994) have also reported that i.c.v. infusion of MIP-1 induced a long-lasting fever. Moreover, it was reported that i.c.v. administration of three members of the CXC chemokines (interleukin-8, platelet factor-4, and interferon-inducible protein-10) and two members of the CC chemokines (monocyte chemotactic protein-1 (MCP-1) and regulated upon activation normal T-cell expressed and presumably secreted (RANTES)) decreased food intake (Carlos et al., 1994). In addition, expression of the chemokines in the brain following stresses was also reported. Sakamoto et al. (1996) have found stress-sensitive CINC expression in hypothalamic nuclei such as the paraventricular nucleus. Furthermore, the authors reported that CINC mRNA expression in the hypothalamus is augmented by osmotic stimulation (Koike et al., 1997). These observations and our present results indicate that chemokines play important roles in the brain, and it is speculated that the chemokines could exert these effect through the chemokinergic neuronal pathway in the CNS.

On the other hand, the types of cells in the brain which express the chemokine receptors remain unclear. Recently, there have been a few reports that some chemokine receptors, including CXCR2 which is the receptor for CINC-1, are expressed in the microglia, astrocyte and neuron (Horuk et al., 1997; Rottman et al., 1997). These data suggest that the hyperalgesic effect of CINC-1 revealed by this study is mediated by CXCR2 expressed in brain.

References

Carlos, R., Salaman, P., Borkoski, J.P., 1994. Chemokines/intercrines and central regulation of feeding. Am. J. Physiol. 266, 1711–1715.

Cunha, F.C., Lorenzetti, B.B., Poole, S., Ferreira, S.H., 1991. Interleukin-8 as a mediator of sympathetic pain. Br. J. Pharmacol. 104, 765–767.

Horuk, R., Martin, A.W., Wang, Z., Schweitzer, L., Gerassimides, A., Guo, H., Lu, Z., Hesselgesser, J., Perez, H.D., Kim, J., Parker, J., Hadley, T.J., Peiper, S.C., 1997. Expression of chemokine receptors by subsets of neurons in the central nervous system. J. Immunol. 158, 2882–2890.

Koike, K., Sakamoto, Y., Kiyama, H., Masuhara, K., Miyake, A., Inoue, M., 1997. Cytokine-induced neutrophil chemoattractant gene expresion in the rat hypothalamus by osmotic stimulation. Mol. Brain Res. 52, 326–329.

Miñano, F.J., Vizcaino, M., Myers, R.D., 1991. Hypothalamic indomethacin fails to block fever induced in rats by central macrophage inflammatory protein-1 (MIP-1). Pharmacol. Biochem. Behav. 39, 535–539.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, Academic Press, Australia.

Petrek, M., Bois, R.M.D., Sírová, M., Weigl, E., 1995. Chemotactic cytokines (chemokines) and their role in physiological and immunopathological reactions. Folia Biologica 41, 263–283.

Rottman, J.B., Ganley, K.P., Williams, K., Wu, L., Mackay, C.R., Ringler, D.J., 1997. Cellular localization of the chemokine receptor CCR5. Am. J. Pathol. 151, 1341–1351.

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, 133–142.

Watanabe, K., Konishi, K., Fujioka, M., Kinoshita, S., Nakagawa, H., 1989a. The neutrophil chemoattractant produced by the rat kidney epithelioid cell line NRK-52E is a protein related to the KC/gro protein. J. Biol. Chem. 264, 19559–19563.

Watanabe, K., Kinoshita, S., Nakagawa, H., 1989b. Purification and characterization of cytokine-induced neutrophil chemoattractant producedby epithelioid cell line of normal rat kidney (NRK-52E cell). Biochem. Biophys. Res. Commun. 161, 1093–1099.

Watanabe, K., Koizumi, F., Kurashige, Y., Tsurufuji, S., Nakagawa, H., 1991. Rat CINC, a member of the interleukin-8 family, is a neutrophil-specific chemoattractant in vivo. Exp. Mol. Pathol. 55, 30–37.

Yabuuchi, K., Nishiyori, A., Minami, M., Satoh, M., 1996. Biphasic effects of intracerebroventricular interleukin-1β on mechanical nociception in the rat. Eur. J. Pharmacol. 300, 59–65.

Yoshimura, T., Matsushima, K., Tanaka, S., Robinson, E.A., Appella, E., Oppenheim, J.J., Leonard, E.J., 1987. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc. Natl. Acad. Sci. USA 84, 9233–9237.

Zagorski, J., Delarco, J.E., 1993. Rat CINC (Cytokine-Induced Neutrophil Chemoattractant) is the homolog of the human GRO proteins but is encoded by a single gene. Biochem. Biophys. Res. Commun. 190, 104–110.

Zawada, W.M., Ruwe, W.D., Clarke, J., Wall, P.T., Myers, R.D., 1994. Chemokine-induced fever: intracerebroventricular action of MIP-1 and MIP-1α in rats. NeuroReport 5, 1365–1368.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.