Tohoku J. Exp. Med., 1999, **189**, 21-27

The Fragment of Complement 3a Lacking the Primary 9 Amino Acids Induces Promoting Activity on Mouse Voluntary Running

YUTAKA MASUDA, NAOYUKI MIURA,[1] MINORU SUZUKI,[2] YUSUKE AKAGAWA,[2] YOSHIHIKO KAWARADA,[1] MASAMI KAWAGOE,[1] TOSHIHIRO SUGIYAMA,[1] TETSUO SHIMIZU[2] and YASUO HISHIKAWA[2]

Psychosomatic Division, Akita University School of Medicine, Akita 010-8543, [1]*Department of Biochemistry, Akita University School of Medicine, Akita 010-8543, and* [2]*Department of Neuropsychiatry, Akita University School of Medicine, Akita 010-8543*

MASUDA, Y., MIURA, N., SUZUKI, M., AKAGAWA, Y., KAWARADA, Y., KAWAGOE, M., SUGIYAMA, T., SHIMIZU, T. and HISHIKAWA, Y. *The Fragment of Complement 3a Lacking the Primary 9 Amino Acids Induces Promoting Activity on Mouse Voluntary Running.* Tohoku J. Exp. Med., 1999, **189** (1), 21-27 —— We have newly found that interleukin-2 (IL-2) increases mouse voluntary running 24 hours, but not 30 minutes, after the injection. We suspected that IL-2 induced a substance increasing the voluntary running for 24 hours after injection. Serum obtained from mice 24 hours after the IL-2 treatment was fractioned with the use of an ion-exchanger and an ultra-filtration method, and the amino acid sequence analysis indicated that the substance purified from the effective fraction was a fragment of mouse complement 3a (C_{3a}) lacking the primary 9 amino acids. The 20 amino acid peptide synthesized according to the fragment showed the activity increasing the voluntary running, but the 20 amino acid peptide synthesized according to the C_{3a} itself did not. The effect of the synthesized peptide was demuted by haloperidol but not by a specific dopamine 2 antagonist (-)sulpiride. The present findings clearly indicate that IL-2 produces the C_{3a} fragment lacking the primary 9 amino acids which directly promotes the voluntary running, and that the effect of the fragment is mediated by an activity of haloperidol on the neurons, except for the dopamine 2 antagonism. ———— interleukin-2; mouse voluntary running; complement 3a; amino acid sequence; haloperidol

Interleukin-2 (IL-2) increases in the process of recovery from an infection, and a mouse shows various behaviors for recovering from an infection. Acute effects of IL-2 on behaviors and neurotransmitters have been reported (Connor et

Received May 24, 1999; revision accepted for publication August 31, 1999.

Address for reprints: Yutaka Masuda, Psychosomatic Division, Akita University School of Medicine, Akita 010-8543, Japan.

al. 1998; Pauli et al. 1998) and the effects of repeated administrations of IL-2 on mouse open-field behaviors has been also reported (Song and Leonard 1995). From the results of these previous reports, we have been interested in relations of IL-2 and mouse escape-directed behaviors from the infectious and harmful circumstances. Here, we have investigated the effect of IL-2 on the voluntary running, and newly found that IL-2 increases the voluntary running 24 hours, but not 30 minutes, after the injection. Although IL-2 directly affects brain function, its half life is below 30 minutes (Zalcman et al. 1994). We suspect that IL-2 induced a substance which increased the voluntary running for 24 hours after injection. In this paper, we describe the detection of the substance and the neurological mechanism of the effect.

Materials and Methods

Animals

The subjects were male ddY mice (SLC Co., Tokyo), 8 weeks of age and weighing 26–28 g. The mice, divided into groups of 5 animals, were housed in plastic cages (338×140×225 mm) with free access of a solid diet (F2: Funabashi Co., Tokyo) and water. The environment was maintained at a temperature of 21–25°C and at a humidity of 50–60% with lights on between 7 : 00 and 19 : 00. The mice in each cage were individually marked for identification. All of the following experiments were approved by the Ethics Committee for Animal Experiments of Akita University School of Medicine, Japan.

Measurement of voluntary running

Mice used in the present studies had been previously placed in a mouse wheel-running apparatus (Natsume Co., Tokyo) and left there for 30 minutes 24 hours before the following experiments. The mice variously treated were placed in the apparatus and the number of wheel revolutions by their voluntary running was counted for 30 minutes. After the counting, the mice were returned to their home cage. The measurement was performed from 15 : 00 to 17 : 00.

Late effect of IL-2 on mouse voluntary running

Mouse IL-2 (Inter-cell Technologies, Hopewell, NJ, USA) was prepared in physiological saline (PS). A group of mice were injected intraperitoneally with 30, 300 or 3000 ng of IL-2, or 100 μl of PS. Immediately or 24 hours after the injection, the group of mice were individually placed in the apparatus and the numbers of the wheel revolutions were counted for 30 minutes.

Serum preparation

Serum was obtained from mice injected with 3000 ng of IL-2 30 minutes or 24 hours after the injection, or mice injected with 100 μl of 0.5% carboxymethylcellulose sodium (CMC; Funakoshi Co.) 24 hours after the injection. Ten ml of each

serum was desalted with the use of a Sephadex G-100 column (Pharmacia Co., Uppsala, Sweden), and the each desalted serum was applied to a DEAE cellulose column (Whatman Co., Maidstone, UK) previously saturated with buffer 10 mM $NaCO_3$, pH 8.3, and was eluted with the buffer, 50, 100, 150, 200, 250 and 300 mM NaCl. Each eluted fraction was divided to above 50 kDa, 50～30 kDa, 30～10 kDa, 10～3 kDa and below 3 kDa with an ultra filtration method (Centricon: Grace Japan Co., Tokyo). The total 35 fractions divided from the each serum were prepared to have 10 mg/ml of protein, and then 200 μl of the prepared fractions were individually injected into 35 groups of mice. The voluntary running of these mice was measured for 30 minutes after the injection.

Detection of the substance increasing the voluntary running

The effective fraction was analyzed with the use of SDS-PAGE method. In this analysis, one peptide band was found in the fraction, and the 20 amino acid sequence of the peptide was analyzed with the use of a protein sequencer (Procise 491: Perkin-Elmer Co., Tokyo). Two different peptides, MDKAGQYTDKGLR-KCCDGMR (the 20 amino acid sequence of the peptide found in the fraction; peptide A) and RSVQLHERRMDKAGQYTDKG (primary 20 amino acid sequence of mouse complement 3a [C_{3a}]; peptide B), were synthesized (Sawadie Technologies Co., Tokyo). A group of mice were injected with 2, 1 or 0.2 mg/kg of the peptide A or 2 mg/kg of the peptide B, or 100 μl of PS, and the voluntary running of these mice was measured for 30 minutes after the injection.

The neurological mechanism of the effect of the peptide A

In order to get further informations of the neurological mechanism of the effect, we investigated the effects of yohimbine (a specific α-2 adrenoceptor antagonist), (-)sulpiride (a specific dopamine 2 receptor antagonist), and haloperidol (a dopamine 2 antagonist) on the activity of the peptide A. Yohimbine, (-) sulpiride or haloperidol (Funakoshi Co., Tokyo) was prepared with 0.5% CMC. In order to determine the maximal sub-effective doses, these antagonists were intraperitoneally injected into groups of mice at various doses and the number of wheel revolutions by voluntary running was counted for 30 minutes after the antagonist injection. Following this, a group of mice were intraperitoneally injected with 2 mg/kg of the peptide A and the each antagonist at the maximal sub-effective or lower doses (yohimbine: 6.3, 12.5, 25 μg/kg, (-)sulpiride: 125, 250, 500 μg/kg, haloperidol: 0.5, 1, 2 μg/kg), or 100 μl of 0.5% CMC. The voluntary running of these mice was measured for 30 minutes after the injection.

Statistical analysis

Analysis of variance (ANOVA) was used to find significant differences among clusters. After the significant differences were found (p values of less than 0.05), Mann-Whitney U-test was used.

TABLE 1. *Late effect of IL-2 on the mouse voluntary running*

Time after injection (hours)	PS (Control)	IL-2 (ng)		
		30	300	3000
0	403.8±9	402.8±8	412.8±13	408.8±12
24	424.2±19	486.2±25	591.2±16**	678.4±34**

A value in this table indicates the mean±S.E. of number of wheel revolutions in 5 mice for 30 minutes. IL-2 did not affect the revolutions at the any doses 0 hour after the injections, but the revolutions of the mice injected with 300 and 3000 ng of IL-2 remarkably increase 24 hours after the injections. (ANOVA; $F=18.2$, $p<0.05$). $^{**}p<0.01$ compared to the control (Mann-Whitney U-test). PS, physiological saline.

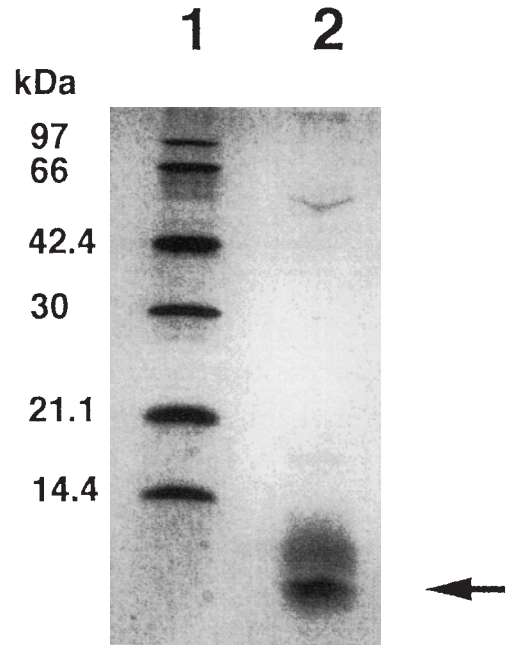

Fig. 1 SDS-PAGE of the peptide in the fraction which increased the voluntary running. Lane 1; molecular weight markers, Lane 2; the fraction. The peptide is indicated by an arrow.

RESULTS

IL-2 increased mouse voluntary running dose-dependently 24 hours, but not 30 minutes, after the injection (Table 1). The one fraction (diluted with the buffer and having 3-10 kDa) divided from the serum obtained from the mice 24 hours after the IL-2 injection showed the voluntary running-increasing activity (the revolution count; 538.4±12), but any fractions divided from the serum obtained from the mice 30 minutes after the (-)sulpiride injection and that obtained from the mice 24 hours after the 0.5% CMC injection did not. The effective fraction contained one peptide with about 10 kDa (Fig. 1) and the amino acid sequence analysis indicated that the peptide had MDKAGQYTDKGLRKCC-DGMR as the 20 primary sequence. This sequence corresponds to that of mouse

TABLE 2. *Effects of the two peptides on the voluntary running*

Dose (mg/kg)		Number of wheel revolution for 30 minutes
Peptide A	2	570.2±21**
	1	519.4±18**
	0.2	462.8±11**
Peptide B	2	403.4± 9
(Control)		
PS	—	399.6±11

Peptide A, MDKAGQYTDKGLRKCCDGMR; Peptide B, RSVQLHERRMDKAGQYTDKG; PS, physiological saline. A value in this table indicates the mean±S.E. of 5 mice. ANOVA any Peptide A ($F=23.1$, $p<0.05$). **$p<0.01$ compared to the control (Mann-Whitney U-test).

TABLE 3. *Effects of the receptor antagonists on the peptide A action in the voluntary running*

Antagonist	Dose (μg/kg)	Counts for 30 minutes after co-injection with 2 mg/kg of peptide A
Yohimbine	25	533.8±20
	12.5	541.4±18
	6.3	549.2±16
(-) Sulpiride	500	543.0±13
	250	546.2±14
	125	544.6±12
Haloperidol	2	429.2±8**
	1	461.4±12**
	0.5	520.0±17
(Control)		
0.5% CMC	—	547.0±22

The drugs were prepared with 0.5% carboxymethylcellulose sodium (CMC). A value in this table indicates mean±S.E. of 5 mice. ANOVA any Haloperidol ($F=13.3$, $p<0.05$). **$p<0.01$ compared to the control (Mann-Whitney U-test).

C_{3a} lacking the primary 9 sequence RSVQLHERR (investigated with the use of DBGET integrated database retrieval system, GenomeNet). Here, the peptide A with the 20 amino acid sequence according to that of the peptide found in the fraction and the peptide B with primary 20 amino acid sequence of C_{3a} itself were synthesized. The peptide A showed the activity increasing the voluntary running with a dose-dependent manner, but the peptide B did not. Although C_{3a} is an anaphylatoxin, irritable behaviors were not found in the mice treated with the peptide A and the peptide B (Table 2). The voluntary running-increasing activity of the peptide A was demuted by haloperidol dose-dependently, but

yohimbine and (-)sulpiride did not affect the activity of the peptide A (Table 3).

Discussion

The present findings clearly indicate the next 3 points; 1) IL-2 induces the fragment of mouse C_{3a} lacking the primary 9 amino acid sequence (RSVQCHER-R) for 24 hours after the injection; 2) the 20 amino acid peptide synthesized according to the fragment (the peptide A) increases mouse voluntary running; 3) the effect of the peptide A is demuted by haloperidol but not by (-)sulpiride.

IL-2 increases C_{3a} production (Brooimans et al. 1991), but the delayed effect of IL-2 on the voluntary running will be induced not only by the increased C_{3a} but also by the C_{3a} catabolism continuing for 24 hours. Although the mechanism of processing C_{3a} into the effective fragment has not been clear, our findings strongly suggest that C_{3a} acts as an internal behavioral activator when it loses the primary 9 amino acids. As the C_{3a} fragment, as well as the peptide A, is a small molecule, it may directly reach the hypothalamus and induce the behavioral effect. A previous report has indicated that mouse locomotion is regulated by α-2 adrenoceptor and dopamine 2 receptor activities (Lin et al. 1983). But, in the present study, effect of the peptide A was demuted by haloperidol but not by a specific dopamine 2 antagonist (-)sulpiride and a specific α-2 adrenoceptor antagonist yohimbine. As it is said that haloperidol has not only dopamine 2 but also δ receptor antagonizing activities, it may be suggested that the effect of the peptide A is mediated by the δ receptor activity. In fact, a previous report has indicated that a haloperidol sensitive δ receptor is detected in the motor nucleus which is involved in voluntary motor behavior (McLean and Weber 1988). But, the nature of the δ receptor has not been clear and it has been reported that monoamine neuron activities are affected by the other opioid receptors (Lin et al. 1983; Schad et al. 1996). Consequently, the neuronal mechanism of the inhibiting effect of haloperidol has not been clear in the present study.

The findings of the present study newly and clearly indicate one of the behavioral functions of the C_{3a} fragment. A previous report has indicated that patients suffering from schizophrenia have genetic abnormalities in the C_{3a} (Fananas et al. 1992). This is also interesting in the relations of C_{3a} and behaviors. Although it is not clear which human behavior corresponds to the mouse voluntary running, our present findings may contribute to the further investigations to the mechanism of the behavioral abnormalities of the patients suffering from schizophrenia.

Acknowledgments

We express our thanks to Dr. Yasuhito Morimoto (Yoshitomi Co., Osaka) for supry of the control mouse serum. We had a financial support from the Ryoichi Naito Foundation for Medical Research.

References

1) Brooimans, R.A, Stegmann, A.P., van-Dorp, W.T., van-der-Ark, A.A., van-der-Woude, F.J., van-Es, L.A. & Daha, M.R. (1991) Interleukin 2 mediates stimulation of complement C_3 biosynthesis in human proximal tubular epithelial cells. *J. Clin. Invest.*, **88**, 379–384.
2) Connor, T.J., Song, C., Leonard, B.E., Merali, Z. & Anisman, H. (1998) An assessment of the effects of central interleukin-1 beta, -2, -6 and tumor necrosis factor-alpha administration on some behavioral, neurochemical, endocrine and immune parameters in the rat. *Neuroscience*, **84**, 926–933.
3) Fananas, L., Moral, P., Panadero, M.A. & Bertranpetit, J. (1992) Complement genetic markers in schizophrenia: C_3, BF and C_6 poly porphisms. *Hum. Hered.*, **42**, 162–167.
4) Lin, M.T., Chan, H.K., Chen, C.F. & The, G.W. (1983) Involvement of both opiate and catacholaminergic receptors in behavioral excitation provoked by thyrotropin-releasing hormone: Comparisons with amphetamine. *Neuropharmacology*, **22**, 463–469.
5) McLean, S. & Weber, E. (1988) Autoradiographic visualization of haloperidol-sensitive sigma receptors in guinea-pig brain. *Neuroscience*, **25**, 256–269.
6) Pauli, S., Linthorst, A.C. & Real, J.M. (1988) Tumor necrosis factor-alpha and interleukin-2 differentially affect hippocampal serotonergic neurotrasmission, behavioral activity, body temperature and hypothalamic-pituitary-adrenocortical axis activity in the rat. *Eur. J. Neurosci*, **10**, 868–878.
7) Schad, C.A., Justice, J.B., Jr. & Holtzman, S.G. (1996) Differential effects of delta- and mu-opioid receptor antagonists on the amphetamine-induced increase in extra cellular dopamine in striatum nucleus accumbens. *J. Neurochem.*, **67**, 2293–2299.
8) Song, C. & Leonard, B.E. (1995) Interleukin-2-induced changes in behavioral, neurotransmitter, and immunological parameters in the factory bulbectomized rat. *Neuroimmnomodulation*, **2**, 263–273.
9) Zalcman, S., Green-Johnson, J.M., Murray, L., Nance, D.M., Dyck, D., Anisman, H. & Greeberg, A.H. (1994) Cytokine-specific central monoamine alteration induced by interleukin-1, -2 and -6. *Brain Res.*, **643**, 40–49.