Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 78 (2004) 799–803

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Effects of the traditional Japanese medicine Unkei-to on the corticotropin-releasing factor–induced increase in locomotor activity

Kiyoshi Terawaki[a,*], Koji Koike[b], Mitsutoshi Yuzurihara[a], Yoshio Kase[a], Shuichi Takeda[a], Masaki Aburada[a], Kouichi Murakami[b], Satoshi Ohno[c], Nobutaka Suzuki[c], Masaki Inoue[b]

[a]*Tsumura Research Institute Medicinal Evaluation Laboratory, Tsumura and Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan*
[b]*Department of Obstetrics and Gynecology, School of Medicine, Kanazawa University, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan*
[c]*Department of Complementary and Alternative Medicine, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8640, Japan*

Received 18 August 2003; received in revised form 18 May 2004; accepted 20 May 2004
Available online 26 June 2004

Abstract

The effect of Unkei-to, a traditional Japanese herbal medicine and strong in vitro releaser of cytokine-induced neutrophil chemoattractant (CINC), on the increase in locomotor activity induced by intracerebroventricular (icv) injection of corticotropin-releasing factor (CRF) in male rats in a familiar environment was investigated. Oral administration of Unkei-to (100 mg/kg) for 1 week significantly attenuated the CRF-induced increase in locomotor activity. Unkei-to also reduced the CRF-induced accumulation of hypothalamic CINC, which has a functional antagonistic action on the response to CRF; the reduction may reflect an increased release of CINC. These results suggest that Unkei-to has an alleviative effect on the action induced by brain CRF and the mechanism of this effect may partly involve CINC.

Keywords: Traditional Japanese medicine; Unkei-to; CINC; CRF; Locomotor activity; Stress

1. Introduction

Traditional Japanese medicine (herbal medicine) has been mainly used for the treatment of chronic diseases. Recently, clinical evidence relating to the curative effects of these medicines has been accumulating and the effectiveness of such treatments has been evaluated using contemporary scientific procedures (Yano et al., 1994; Enomoto et al., 1992; Usuki, 1991). Unkei-to, one of the most popular herbal medicines in Japan, is manufactured from 12 medicinal herbs. Unkei-to is generally used to treat women who are suffering from irregular menstruation, sterility, and climacteric disturbances (Koyama et al., 1988; Ushiroyama et al., 1995). Animal studies showed that Unkei-to acts on the endocrine system such as the hypothalamus–pituitary–ovarian axis (Miyake et al., 1986; Koike et al., 1998b; Yasui et al., 2003).

There is a growing body of evidence that the neuroendocrine and immune systems are engaged in a functionally relevant crosstalk with each other (Berkenbosch et al., 1987; Bernton et al., 1987). These interactions are also directly linked to the stress system. Corticotropin-releasing factor (CRF) is released from the hypothalamus and plays a key role in the stress system. Intracerebroventricular (icv) injection of CRF induces a variety of behavioral manifestations similar to a state of stress (Sutton et al., 1982; Britton et al., 1982; Sherman and Kalin, 1988). We also confirmed that icv injection of CRF significantly increased locomotor activity in a familiar environment (Terawaki et al., 2001a). Thus, CRF also has extrahypophysiotropic functions and the brain CRF system plays an important role in mediating behavioral responses to stressors in addition to activation of the hypothalamus–pituitary–adrenal axis during stress.

One of the chemokines is cytokine-induced neutrophil chemoattractant (CINC), which exhibits high homology with chemokines such as growth-related oncogene (GRO) product and human IL-8 in amino acid sequence (Watanabe et al., 1989). Interestingly, Unkei-to potently stimulated the

* Corresponding author. Tel.: +81-29-889-3859; fax: +81-29-889-2158.
E-mail address: terawaki_kiyoshi@mail.tsumura.co.jp (K. Terawaki).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.05.012

release of CINC from rat anterior pituitary cells and TtT/GF cells (Koike et al., 1998a). Furthermore, we found the presence of a CINC neuronal pathway in the hypothalamo–pituitary system, which was activated in response to various kinds of stress such as endotoxin (Sakamoto et al., 1996a), immobilization stress (Sakamoto et al., 1996b), and insulin-induced hypoglycemia (Osako et al., 1999). We also found that CINC injected icv significantly reduced the enhanced locomotor activity induced by CRF, although a similar effect was not observed following peripheral administration of CINC at a dose sufficient to induce the chemotactic activity of neutrophils (Terawaki et al., 2001a), suggesting that CINC in the brain, but not in the peripheral circulation, may attenuate the responses to CRF.

These observations led us to investigate the effect of Unkei-to on the CRF-induced increase in locomotor activity in a familiar environment. We further examined changes of CINC concentration in the hypothalamus, an important brain region for production of the CRF-induced increase in locomotor activity (Mönnikes et al., 1992).

2. Materials and methods

2.1. Animals

All animal experiments were performed in accordance with our institutional guidelines after obtaining the permission of the Laboratory Animal Committee. Six-week-old male Sprague–Dawley rats were obtained from Charles River Laboratories (Yokohama, Japan). All animals were housed at 23 ± 2 °C with a 12-h light/dark cycle (lights on at 7:00 a.m.) for at least 1 week before starting the experiments. Food and water were provided ad libitum.

2.2. Drugs

Human/rat corticotropin-releasing factor (CRF) was purchased from Peptide Institute (Osaka, Japan) and dissolved with 0.1% acetic acid. On the day of experiments, CRF was diluted with phosphate-buffered saline (PBS) at a concentration of 0.25 nmol/5 μl. Ethylenediaminetetraacetic acid disodium salts (EDTA 2Na), benzamidine hydrochloride (BZ), and leupeptin hydrochloride (LP) were purchased from Sigma (St. Louis, MO, USA), while (*p*-amidinophenyl) methanesulfonyl fluoride hydrochloride (AMSF) was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Unkei-to is composed of 12 medicinal herbs in fixed proportions: *Ophiopgonis tuber* 4.0 g, *Pinelliae tuber* 4.0 g, *Angelicae radix* 3.0 g, *Glycyrrhizae radix* 2.0 g, *Cinnamomi cortex* 2.0 g, *Paeoniae radix* 2.0 g, *Cnidii rhizome* 2.0 g, *Ginseng radix* 2.0 g, *Moutan cortex* 2.0 g, *Evodiae fructus* 1.0 g, *Zingiberis rhizome* 1.0 g, and *Asini Corii Collas* 2.0 g. The drugs were prepared as a spray-dried powder from a hot-water extract and obtained from Tsumura (Tokyo, Japan). Unkei-to was dissolved in distilled water. Unkei-to was compulsively administered to the inside of the stomach using a plastic probe at 13:00–15:00 h once a day.

2.3. Surgery

Animals were anesthetized with sodium pentobarbital (50 mg/kg ip), then placed in a stereotaxic instrument (SR-5; NARISHIGE, Tokyo, Japan) and secured with rat ear bars. A stainless steel guide cannula (AG-8; Eicom, Kyoto, Japan) was implanted stereotaxically 0.8 mm posterior from the bregma and 1.2 mm right lateral from the middle suture. The injection depth was 3.8 mm from the skull surface. The cannula was secured to the skull with two stainless steel screws and dental cement. The guide cannula was protected with a capnut and dummy cannula. Animals were allowed to recover for 1 week before experiments. The day before the locomotor test, rats were placed with food and water in experimental Plexiglas cages ($30 \times 40 \times 40$ cm) to allow overnight habituation to their environment.

2.4. Locomotor activity

Unkei-to (10, 30, and 100 mg/kg) or distilled water (10 ml/kg) was administered orally for 1 week until the day before the experiments. On the day of the experiments, CRF (0.25 nmol/5 μl) or PBS (5 μl) was perfused at a flow rate of 1.0 μl/min using a microsyringe. After each icv injection, the injection cannula was left in place for 1 min. Locomotor activity was monitored for 180 min immediately after icv injections using infrared sensors (NS-AS01; Neuroscience, Tokyo, Japan) placed on the top of an experimental cage. Data on locomotor activity were collected and analyzed with a DAS interface unit NS-DAS-8 (Neuroscience) and a personal computer (NEC, Tokyo, Japan) equipped with the DAS system (Neuroscience) data recording software. After the experiments, cannula placements were verified by injecting local dye.

2.5. Measurement of CINC concentration in the hypothalamus

Unkei-to at a dose of 100 mg/kg or distilled water was administered orally to rats for 1 week until the day before experiments. The brain was removed before or 30 min after CRF icv injection. The hypothalamus was dissected stereotaxically by cutting the brain on ice-chilled sterilized glass boards, at the optic chiasma rostrally and caudally, at 2.5 mm from the midline laterally, and at 3 mm from the base of the brain dorsally, and the wet weight was measured. These tissues were stored at −80 °C until the time of use.

On the day of the assay, each tissue was homogenized in 0.5 ml of PBS containing enzyme inhibitors (5.0 mM BZ,

10 μg/ml LP, 0.2 mM AMSF, and 10 mM EDTA 2Na) using a microhomogenizer. Furthermore, these samples were sonicated using an ultrahomogenizer (SMT, Tokyo, Japan) for 10 s. The homogenates were centrifuged at 10,000 × *g* and 4 °C, for 10 min. The supernatants were used for determination of the CINC concentration and total protein. The determinations of protein concentration were carried out according to the Lowry method (Lowry et al., 1951). The CINC concentration was determined using a rat CINC enzyme immunoassay (EIA) kit (Immuno-Biological Laboratories, Gunma, Japan).

2.6. Statistics

The results are expressed as the mean ± S.E.M. Data for the time course of locomotor activity were analyzed using a two-factor repeated measures analysis of variance (ANOVA). The differences in total counts of locomotor activity between groups were evaluated using a one-factor measures ANOVA followed by the post hoc Dunnett's test. Hypothalamic CINC levels were analyzed using a two-factor ANOVA and the differences between groups were evaluated by the Mann–Whitney *U* test. $P < .05$ was considered significant.

3. Results

3.1. Effect of oral Unkei-to pretreatment on the CRF-induced increase in locomotor activity

Time course changes in locomotor activity following icv injection of PBS or CRF in rats pretreated with distilled water (D.W.) are shown in Fig. 1. The injection of PBS induced a rapid decrease in locomotor activity,

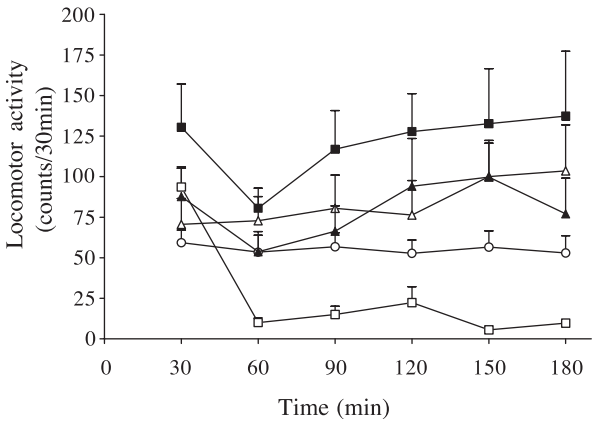

Fig. 1. Time course of the effects of Unkei-to administered orally for 1 week on the increase in locomotor activity induced by icv injection of CRF at 0.25 nmol/animal in male rats in a familiar environment. Locomotor activity was monitored for 180 min immediately after the injections using infrared sensors. Each point represents the mean ± S.E.M. of 8–10 rats. (□—□) D.W. + PBS icv; (■—■) D.W. + CRF icv; (△—△) Unkei-to (10 mg/kg) + CRF icv; (▲—▲) Unkei-to (30 mg/kg) + CRF icv; (○—○) Unkei-to (100 mg/kg) + CRF icv.

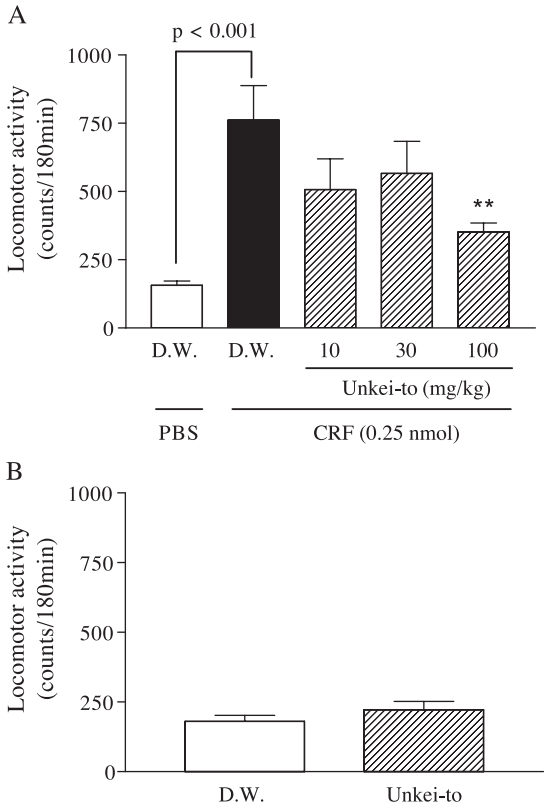

Fig. 2. (A) Effects of Unkei-to administered orally for 1 week on the increase in locomotor activity induced by icv injection of CRF at 0.25 nmol/animal in male rats in a familiar environment. Locomotor activity was monitored for 180 min immediately after the injections using infrared sensors. Each point represents the mean ± S.E.M. of 8–10 rats. $^{**}P < .01$ vs. D.W. + CRF icv. (B) Effect of Unkei-to (100 mg/kg) administered orally for 1 week on locomotor activity following icv injection of PBS in male rats in a familiar environment. Locomotor activity was monitored for 180 min immediately after the injections using infrared sensors. Each point represents the mean ± S.E.M. of 9–10 rats.

which reached minimal levels by 60 min. In contrast, the injection of CRF produced a continuous increase in locomotor activity up to 180 min, the end of the period of measurement. The time-course curves between these groups was significantly different [$F(1,70) = 22.617$, $P < .001$]. There was also a significant group × time interaction between them [$F(5,70) = 2.367$, $P < .05$].

On the other hand, pretreatment with Unkei-to (100 mg/kg) attenuated the CRF-induced increase in locomotor activity throughout this period and there was a significant difference in the group factor between the D.W. + CRF icv group and Unkei-to (100 mg/kg) + CRF icv group [$F(1,70) = 10.218$, $P < .01$], but not in group × time interaction. Fig. 2A shows the changes in each group as total counts for 180 min of locomotor activity induced by the injection of CRF. Unkei-to at a dose of 100 mg/kg significantly reduced the increase in locomotor activity induced by CRF. However, similar pretreatment with Unkei-to did not affect locomotor activity with icv injection of PBS (Fig. 2B).

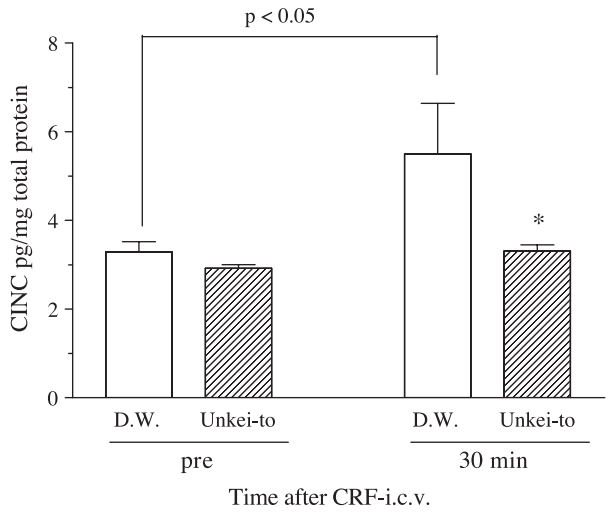

Fig. 3. Effects of Unkei-to (100 mg/kg) administered orally for 1 week on the levels of hypothalamic CINC 30 min after icv injection of CRF (0.25 nmol). Data are expressed as the mean ± S.E.M. of results from 8 to 10 rats. * $P < .05$ vs. D.W., 30-min group.

3.2. Effect of Unkei-to administration on the hypothalamic CINC concentration

Effects of Unkei-to on the hypothalamic CINC concentration in CRF-injected rats are shown in Fig. 3. The hypothalamic CINC concentration in D.W.-treated rats was significantly increased 30 min after the injection compared to that before injection, but not in Unkei-to–treated rats. There was a significant difference between time-course curves in these two groups [$F(1,36) = 4.749$, $P < .05$]. The hypothalamic CINC concentration in Unkei-to–treated rats 30 min after the injection was significantly reduced compared to that in D.W.-treated rats.

4. Discussion

This study showed that oral administration of Unkei-to for 1 week attenuated the CRF-induced increase in locomotor activity, as did icv injection of CINC, which has a functional antagonistic action on the CRF-induced increase in locomotor activity (Terawaki et al., 2001a). It was also confirmed that the same treatment with Unkei-to did not affect the time course or total counts of locomotor activity following icv injection of PBS in this study, suggesting that the effect of Unkei-to on the CRF-induced increase in locomotor activity is not due to sedative actions or muscle relaxation actions such as those exhibited by haloperidol and dantrolene (Hammond et al., 1991). In addition to CRF as a drug producing the increase in locomotor activity, psychostimulants such as amphetamine and cocaine, or caffeine are known (Swerdlow et al., 1986). Especially of these drugs, the cocaine-induced increase in locomotor activity has been suggested to be partly mediated by CRF and therefore may be reduced by Unkei-to (Sarnyai et al., 1992). It is thought that brain CRF plays an important role in the development of stress-related disorders such as anxiety, depression, and irregular menstruation (Magiakou et al., 1997; Stratakis and Chrousos, 1995). Furthermore, it is well known that the antagonistic action against CRF reduced physiological and behavioral changes induced by not only icv injection of CRF but also stress (Heinrichs et al., 1992; Menzaghi et al., 1994). Taken together, it was suggested that Unkei-to may alleviate etiological changes derived from stress. Actually, we indicated that oral administration of Unkei-to significantly improved estrous cycle disturbance induced by forced running stress in experimental animals (Terawaki et al., 2001b). Furthermore, we recently found that treatment with Unkei-to for 3 months significantly improved both anxiety and depression scores in postmenopausal women (manuscript submitted). The alleviation of CRF actions by Unkei-to as observed in the present study may be partly involved in the pharmacological and clinical effects of Unkei-to mentioned above.

When examined whether CINC is involved in the effect of Unkei-to, it was observed that Unkei-to pretreatment for 1 week significantly reduced the CRF-induced accumulation of hypothalamic CINC within 30 min of the injection. It was conceivable that the reduction in hypothalamic CINC concentrations occurred as the result of the release of CINC from hypothalamic cells. If so, it is suggested that CINC plays an important role in the effect of Unkei-to on the CRF-induced increase in locomotor activity. We demonstrated that Unkei-to stimulated the release of CINC and increased CINC mRNA levels in a pituitary folliculu-stellate (FS)-like cell line (Koike et al., 1998a). It has been reported that FS cells function in phagocytosis, ion transport regulation, and a paracrine role, and contain S-100 protein and many lysosomes, similar to glial cells (Matsumoto et al., 1993). Uehara et al. (1998) showed the production of CINC in rat glioma cells. From these findings, it is possible that Unkei-to acts on the hypothalamus and stimulates hypothalamic cells to release CINC. Furthermore, we have confirmed a decrease in the content of CINC as well as increase in release and mRNA in pituitary cells caused by Unkei-to. These observations support the possibility that CINC is released from the hypothalamus by Unkei-to.

Alternatively, the stress-sensitive CINC production may have decreased because Unkei-to attenuated the actions of CRF. Our previous reports have showed that in the hypothalamo–pituitary, the production of CINC was induced in response to various kinds of stress (Sakamoto et al., 1996a,b; Osako et al., 1999). Pretreatment with CRF antagonists reduced the physiological and behavioral changes induced by not only icv injection of CRF but also stress (Heinrichs et al., 1992; Menzaghi et al., 1994). Therefore, it is supposed that the antagonism of the response to CRF reduced hypothalamic CINC production or release. Besides, neuropeptide Y (NPY) exhibited antagonistic activity against CRF-induced responses (Britton et al., 2000). However, it is unclear at present whether Unkei-to has antago-

nistic actions against the binding to CRF receptors or the actions to produce neuropeptides such as NPY.

In conclusion, oral administration of Unkei-to attenuated the CRF-induced increase in locomotor activity. The mechanism of this effect of Unkei-to remains unclear, but could involve hypothalamic CINC, which has a functional antagonistic action on the response to CRF. Clinical curative effects of Unkei-to on stress-related disorders such as irregular menstruation and climacteric disturbances may be partly mediated by the alleviative effect on the actions of CRF.

References

Berkenbosch F, van Oers J, del Rey A, Tilders F, Besedovsky H. Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. Science 1987;238:524–6.

Bernton EW, Beach JE, Holaday JW, Smallridge RC, Fein HG. Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. Science 1987;238:519–21.

Britton DR, Koob GF, River J, Vale W. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. Life Sci 1982;31:363–7.

Britton KT, Akwa Y, Spina MG, Koob GF. Neuropeptide Y blocks anxiogenic-like behavioral activation of corticotropin-releasing factor in an operant conflict test and elevated plus maze. Peptides 2000;21:37–44.

Enomoto K, Higashida H, Maeno T. Effects of toki-shakuyaku-san (Tsumura TJ-23) on electrical activity in neuroblastoma cell and frog neuromuscular junctions. Neurosci Res 1992;15:81–9.

Hammond EO, Torok ML, Ettenberg A. Different patterns of behavior produced by haloperidol, pentobarbital, and dantrolene in tests of unconditioned locomotion and operant responding. Psychopharmacology 1991;104:150–6.

Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF. Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Res 1992;581:190–7.

Koike K, Zhang ZX, Sakamoto Y, Jikihara H, Masuhara K, Murakami K. The Herbal medicine Unkei-to stimulates CINC production in the pituitary folliculo-stellate (FS)-like cell line (TtT/GF). Am J Reprod Immunol 1998a;39:249–55.

Koike K, Zhang ZX, Sakamoto Y, Miyake A, Inoue M. Evidence that folliculo-stellate cells mediate the inhibitory effect of Japanese kampo medicine, Unkei-to, on growth hormone secretion in rat anterior pituitary cell cultures. Am J Reprod Immunol 1998b;39:217–22.

Koyama T, Ohara M, Ichimura M, Saito M. Effect of Japanese Kampo medicine on hypothalamic–pituitary–ovarian function in women with ovarian insufficiency. Am J Chin Med 1988;16:47–55.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.

Magiakou MA, Mastrorakos G, Webster E, Chrousos GP. The hypothalamus–pituitary–adrenal axis and the female reproductive system. Ann N Y Acad Aci 1997;816:42–56.

Matsumoto H, Koyama C, Sawada T, Koike K, Hirota K, Miyake A, et al. Pituitary follicullo-stellate-like cell line (TtT/GF) responds to novel hypophysiotropic peptide (pituitary adenylate cyclase-activating peptide), showing increased adenosine 3′ 5′ -monophosphate and interleukin-6 secretion and cell proliferation. Endocrinology 1993;133:2155–9.

Menzaghi F, Howard RL, Heinrichs SC, Vale W, River J, Koob GF. Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. J Pharmacol Exp Ther 1994;269:564–72.

Miyake A, Lee JW, Tasaka K, Ohtsuka S, Aono T. Wen-jing-tang, a traditional Chinese herbal medicine increases luteinizing hormone release in vitro. Am J Chin Med 1986;14:157–60.

Mönnikes H, Mönnikes IH, Taché Y. CRF in the paraventricular nucleus of the hypothalamus induces dose-related behavioral profile in rats. Brain Res 1992;574:70–6.

Osako Y, Koike K, Kiyama H, Sakamoto Y, Masuhara K, Segawa T, et al. Insulin-induced hypoglycemia activates a chemokinergic neuronal pathway in the hypothalamo–pituitary system. Neuropeptides 1999;33:271–5.

Sakamoto Y, Koike K, Kiyama H, Konishi K, Watanabe K, Osako Y, et al. Endotoxin activates a chemokinergic neuronal pathway in the hypothalamo–pituitary system. Endocrinology 1996a;137:4503–6.

Sakamoto Y, Koike K, Kiyama H, Konishi K, Watanabe K, Tsurufuji S, et al. A stress-sensitive chemokinergic neuronal pathway in hypothalamo–pituitary system. Neuroscience 1996b;75:133–42.

Sarnyai Z, Höhn J, Szabó G, Penke B. Critical role of endogenous corticotropin-releasing factor (CRF) in the mediation of the behavioral action of cocaine in rats. Life Sci 1992;51:2019–24.

Sherman JE, Kalin NH. ICV-CRH alters stress-induced freezing behavior without affecting sensitivity. Pharmacol Biochem Behav 1988;30:801–7.

Stratakis CA, Chrousos GP. Neuroendocrinology and pathophysiology of the stress. Ann N Y Acad Sci 1995;771:1–18.

Sutton RE, Koob GF, Le Moal M, River J, Vale W. Corticotropin releasing factor produces behavioral activation in rats. Nature 1982;297:331–3.

Swerdlow NR, Vaccarino FJ, Amalric M, Koob GF. The neural substrates for the motor-activating properties of psychostimulants: a review of recent findings. Pharmacol Biochem Behav 1986;25:233–48.

Terawaki K, Koike K, Yuzurihara M, Kurauchi K, Ishige A, Sasaki H, et al. An inhibitory effect of cytokine-induced neutrophil chemoattractant on corticotropin-releasing factor-induced increase in locomotor activity. Brain Res 2001a;917:133–7.

Terawaki K, Yuzurihara M, Kurauchi K, Ishige A, Sasaki H. Effects of Unkei-to on the disorder of estrous cycle induced by stress. J Jpn Assoc Orient Psychosom Med 2001b;16:5–9.

Uehara T, Baba I, Nomura Y. Induction of cytokine-induced neutrophil chemoattractant in response to various stresses in rat C6 glioma cells. Brain Res 1998;790:284–92.

Ushiroyama T, Tsubokura S, Ikeda A, Ueki M. The effect of Unkei-to on pituitary gonadotropin secretion and ovulation in anovulatory cycles of young women. Am J Chin Med 1995;23:223–30.

Usuki S. Effect of Hachimijiogan, Tokishakuyakusan, Keishibukuryogan, Ninjinto, and Unkeito on estrogen and progesterone secretion in preovulatory follicles incubated in vitro. Am J Chin Med 1991;19:65–71.

Watanabe K, Konishi K, Nakagawa H. 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 1989;264:19559–63.

Yano H, Mizoguchi A, Fukuda K, Haramaki M, Ogasawara S, Momosaki S, et al. The herbal medicine Sho-saiko-to inhibits proliferation of cell lines by inducing apoptosis and arrest at the G0/G1 phase. Cancer Res 1994;54:448–54.

Yasui T, Matsuzaki T, Ogata R, Kiyokawa M, Ushigoe K, Uemura H, et al. The herbal medicine Unkei-to stimulates the secretion of a cytokine-induced neutrophil chemoattractant, CINC/gro, in the rat ovarian cell culture. Am J Reprod Immunol 2003;49:14–20.