the seven TCI dimensions was significantly associated with cortisol reactivity; lower cooperativeness was related to more suppression of cortisol. In the multivariate analysis, however, the combination of cooperativeness and reward dependence emerged as the most powerful predictor of coritsol reactivity. The latter finding indicates that the combination of reward dependence and cooperativeness, compared to any single personality dimension, better defines a subtype of depression closely linked to altered HPA axis function. This finding also suggests that MDD as defined in the DSM-IV consists of a wide diversity of subtypes and thus the heterogeneity of depression represents a rather complex issue, as has been widely debated (e.g., Ostergaard et al., 2011).

The association between the unique personality profile and blunted cortisol reactivity observed here would be of interest in light of a number of previous studies relating a variety of psychopathologies to hypocortisolism. For example, Tops et al. (2008) found that higher rejection sensitivity was related to salivary lower cortisol awakening response. Depressed patients with long-term sick-leave are shown to display blunted cortisol responses to the DEX/CRH test (Rydmark et al., 2006; Wahlberg et al., 2009). This finding mirrors the observation of Wirtz et al. (2010) that higher overcommitment to work was associated with higher cortisol responses to the DEX/CRH test. Furthermore, O'Leary et al. (2010) reported that non-clinical students characterized by high psychopathic personality traits lacked psychosocial stress-induced cortisol increases. Concordant with this finding, several studies have found non-clinical schizotypal traits to be associated with enhanced cortisol suppression to pharmacological challenge paradigms (Schweitzer et al., 2001; Hori et al., 2011a). Concerning the natural course and meaning of hypocortisolism, however, there remains much to be elucidated. This extremely low cortisol (re)activity can represent the result of prolonged stress exposure (Heim et al., 2000; Fries et al., 2005) while it is also possible that this state could be a preexisting vulnerability to stress-related disorders (Delahanty et al., 2000; Yehuda et al., 2000; Wahlberg et al., 2009); these two possibilities are not mutually exclusive.

With regard to the different cortisol indices in the DEX/CRH test, significant relationships were observed between personality dimensions and reactive cortisol indices to the CRH challenge following the DEX administration (i.e., DEX/CRH-Cort and suppression pattern), but not the cortisol level after the DEX administration (i.e., DST-Cort). In line with the present results, previous studies have consistently found that cortisol levels after the combined DEX/CRH challenge, but not those after the administration of DEX alone, are associated with altered personality profiles (Rinne et al., 2002; Tyrka et al., 2006, 2008; Hori et al., 2011a). Although we can only speculate on the underlying mechanism, these findings suggest that the HPA axis alteration in relation to unique personality makeup would be accounted for, at least in part, by the altered regulation of HPA axis function by hypothalamic CRH (and possibly arginine vasopressin) system rather than by the glucocorticoid receptor-mediated negative feedback inhibition as measured by the DST.

The present findings may be somehow related to the following fact. In Japan, the past decade has seen a dramatic increase in a new type of depression, called modern type of depression. A shift in lifestyle caused by the rapid socioeconomic change is considered responsible for this. Corresponding to this, a survey shows that the number of depressed patients presented to psychiatric clinics/hospitals has nearly doubled during the same time period (Ministry of Health, Labour and Welfare, 2012). A recent international survey shows that this phenomenon is seen in other countries as well, particularly in urban areas (Kato et al., 2011). Although the underlying biological causes and mechanisms, including their neuroendocrine status, have not been well documented, the modern type of depression is usually characterized by their personality traits (Tarumi and Kanba, 2005). Indeed, conceptualization of modern type of depression includes a unique set of personality makeup characterized by attachment to oneself, less loyalty to rules and norms imposed by society with negative feelings toward them, and vague sense of omnipotence (Tarumi and Kanba, 2005; Kato et al., 2011). It is generally acknowledged that the modern type of depression has some phenomenological overlap with atypical depression and dysthymic disorder (Tarumi and Kanba, 2005; Kato et al., 2011). In the present study blunted cortisol reactivity was associated with lower cooperativeness, especially lower social acceptance and compassion. Since this type of personality trait corresponds to the one seen in modern type of depression, our finding points to the possibility that modern type of depression is associated with hypocortisolism, as is the case with atypical depression (Gold and Chrousos, 2002). In contrast, personality in melancholic depression has been traditionally characterized as diligent and hardworking, being loyal to the rules and orders imposed by society and community with positive feelings toward them (Shimoda, 1957; Tellenbach, 1961). Such a personality trait corresponds well to the present combination of higher cooperativeness and lower reward dependence, which in turn was associated with hypercortisolism. Since optimal treatment strategies are likely to be different between melancholic depression and modern type of depression (Tarumi and Kanba, 2005), the present findings could be of clinical importance. Future studies are warranted to define modern type of depression in terms of its underlying biology, including HPA axis function, as well as phenomenology.

The current findings should be considered in the context of a number of limitations. First, since the DEX/CRH test used here was based on a simple test protocol (i.e., measuring the cortisol level only twice), it may have provided less information on HPA axis function (e.g., lack of the ACTH data). Moreover, we did not measure baseline cortisol levels, i.e., the cortisol level before the DEX administration, which prevented us from delineating the extent to which each participant suppressed his/her cortisol in response to the 1.5 mg of DEX. Second, this cross-sectional study does not provide information as to the causality between personality traits and alteration in HPA axis function. Third, we did not collect data on the menstrual cycle or menopausal status in our female participants. While these factors may have influenced their HPA axis function, it is unlikely that the menstrual cycle had any impact on the TCI results. Finally, since most of our patients were receiving psychotropics, such medication may have influenced HPA axis function. We would like to note, however, that the two suppressor groups did not significantly differ in any classes of psychotropic medications (Table 1).

In conclusion, the present findings indicate that there could be a significant variation in cortisol reactivity within depression and thus might point to the importance of taking account of hypocortisolism as well as hypercortisolism. Our findings might also suggest the possibility of differentiating personality-related subtypes of depression based on the different pattern of cortisol reactivity. As this is the first study to examine the relationship of temperament and character with cortisol reactivity to the DEX/CRH test in depressed patients, further studies, ideally in various ethnic groups, are required to replicate these findings.

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study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

Conflict of interest

All authors declare no conflict of interest.

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References

Akiskal, H.S., Kilzieh, N., Maser, J.D., Clayton, P.J., Schettler, P.J., Traci Shea, M., Endicott, J., Scheftner, W., Hirschfeld, R.M., Keller, M.B., 2006. The distinct temperament profiles of bipolar I, bipolar II and unipolar patients. Journal of Affective Disorders 92, 19–33.

American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders, fourth ed. American Psychiatric Association, Washington, DC.

Arana, G.W., Baldessarini, R.J., Ornsteen, M., 1985. The dexamethasone suppression test for diagnosis and prognosis in psychiatry. Commentary and review. Archives of General Psychiatry 42, 1193–1204.

Bagby, R.M., Young, L.T., Schuller, D.R., Bindseil, K.D., Cooke, R.G., Dickens, S.E., Levitt, A.J., Joffe, R.T., 1996. Bipolar disorder, unipolar depression and the five-factor model of personality. Journal of Affective Disorders 41, 25–32.

Bagby, R.M., Psych, C., Quilty, L.C., Ryder, A.C., 2008. Personality and depression. Canadian Journal of Psychiatry 53, 14–25.

Braddock, L., 1986. The dexamethasone suppression test. Fact and artefact. The British Journal of Psychiatry 148, 363–374.

Brändström, S., Richter, J., Przybeck, T., 2001. Distributions by age and sex of the dimensions of temperament and character inventory in a cross-cultural perspective among Sweden Germany, and the USA. Psychological Reports 89, 747–758.

Carpenter, L.L., Ross, N.S., Tyrka, A.R., Anderson, G.M., Kelly, M., Price, L.H., 2009. Dex/CRH test cortisol response in outpatients with major depression and matched healthy controls. Psychoneuroendocrinology 34, 1208–1213.

Carroll, B.J., Feinberg, M., Greden, J.F., Tarika, J., Albala, A.A., Haskett, R.F., James, N.M., Kronfol, Z., Lohr, N., Steiner, M., de Vigne, J.P., Young, E., 1981. A specific laboratory test for the diagnosis of melancholia. Standardization, validation, and clinical utility. Archives of General Psychiatry 38, 15–22.

Carroll, B.J., 1982. The dexamethasone suppression test for melancholia. The British Journal of Psychiatry 140, 292–304.

Ceulemans, D.L., Westenberg, H.G., van Praag, H.M., 1985. The effect of stress on the dexamethasone suppression test. Psychiatry Research 14, 189–195.

Chopra, K.K., Bagby, R.M., Dickens, S., Kennedy, S.H., Ravindran, A., Levitan, R.D., 2005. A dimensional approach to personality in atypical depression. Psychiatry Research 134, 161–167.

Cloninger, C.R., 1987. A systematic method for clinical description and classification of personality variants. A proposal. Archives of General Psychiatry 44, 573–588.

Cloninger, C.R., Przybeck, T.R., Svrakic, D.M., 1991. The tridimensional personality questionnaire: U.S. normative data. Psychological Reports 69, 1047–1057.

Cloninger, C.R., Svrakic, D.M., Przybeck, T.R., 1993. A psychobiological model of temperament and character. Archives of General Psychiatry 50, 975–990.

Delahanty, D.L., Raimonde, A.J., Spoonster, E., 2000. Initial posttraumatic urinary cortisol levels predict subsequent PTSD symptoms in motor vehicle accident victims. Biological Psychiatry 48, 940–947.

Derogatis, L.R., Lipman, R.S., Rickels, K., Uhlenhuth, E.H., Covi, L., 1974. The Hopkins Symptom Checklist (HSCL): a self-report symptom inventory. Behavioral Science 19, 1–15.

Enns, M.W., Cox, B.J., 1997. Personality dimensions and depression: review and commentary. Canadian Journal of Psychiatry 42, 274–284.

Fink, M., 2005. Should the dexamethasone suppression test be resurrected? Acta Psychiatrica Scandinavica 112, 245–249.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J., 1997. User's Guide for the Structured Clinical Interview for DSM-IV Axis I Disorders. Biometrics Research Department. Columbia University, New York, NY.

Fries, E., Hesse, J., Hellhammer, J., Hellhammer, D.H., 2005. A new view on hypocortisolism. Psychoneuroendocrinology 30, 1010–1016.

Gervasoni, N., Bertschy, G., Osiek, C., Perret, G., Denis, R., Golaz, J., Rossier, M.F., Bondolfi, G., Aubry, J.M., 2004. Cortisol responses to combined dexamethasone/CRH test in outpatients with a major depressive episode. Journal of Psychiatric Research 38, 553–557.

Gold, P.W., Chrousos, G.P., 2002. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Molecular Psychiatry 7, 254–275.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. Br. Journal of Social and Clinical Psychology 6, 278–296.

Heim, C., Ehlert, U., Hellhammer, D.H., 2000. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 25, 1–35.

Herbert, J., Ban, M., Brown, G.W., Harris, T.O., Ogilvie, A., Uher, R., Craig, T.K., 2012. Interaction between the BDNF gene Val/66/Met polymorphism and morning cortisol levels as a predictor of depression in adult women. The British Journal of Psychiatry 201, 313–319.

Heuser, I., Yassouridis, A., Holsboer, F., 1994a. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. Journal of Psychiatric Research 28, 341–356.

Heuser, I.J., Gotthardt, U., Schweiger, U., Schmider, J., Lammers, C.H., Dettling, M., Holsboer, F., 1994b. Age-associated changes of pituitary-adrenocortical hormone regulation in humans: importance of gender. Neurobiology of Aging 15, 227–231.

Holsboer, F., von Bardeleben, U., Wiedemann, K., Müller, O.A., Stalla, G.K., 1987. Serial assessment of corticotropin-releasing hormone response after dexamethasone in depression. Implications for pathophysiology of DST nonsuppression. Biological Psychiatry 22, 228–234.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477–501.

Hori, H., Ozeki, Y., Teraishi, T., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Suto, S., Terada, S., Higuchi, T., Kunugi, H., 2010. Relationships between psychological distress, coping styles, and HPA axis reactivity in healthy adults. Journal of Psychiatric Research 44, 865–873.

Hori, H., Teraishi, T., Ozeki, Y., Hattori, K., Sasayama, D., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Higuchi, T., Kunugi, H., 2011a. Schizotypal personality in healthy adults is related to blunted cortisol responses to the combined dexamethasone/corticotropin-releasing hormone test. Neuropsychobiology 63, 232–241.

Hori, H., Teraishi, T., Sasayama, D., Ozeki, Y., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Hattori, K., Higuchi, T., Kunugi, H., 2011b. Poor sleep is associated with exaggerated cortisol response to the combined dexamethasone/CRH test in a non-clinical population. Journal of Psychiatric Research 45, 1257–1263.

Ising, M., Künzel, H.E., Binder, E.B., Nickel, T., Modell, S., Holsboer, F., 2005. The combined dexamethasone/CRH test as a potential surrogate marker in depression. Progress in Neuro-Psychopharmacology & Biological Psychiatry 29, 1085–1093.

Ising, M., Horstmann, S., Kloiber, S., Lucae, S., Binder, E.B., Kern, N., Künzel, H.E., Pfennig, A., Uhr, M., Holsboer, F., 2007. Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression—a potential biomarker? Biological Psychiatry 62, 47–54.

Joyce, P.R., Mulder, R.T., McKenzie, J.M., Luty, S.E., Cloninger, C.R., 2004. Atypical depression, atypical temperament and a differential antidepressant response to fluoxetine and nortriptyline. Depression and Anxiety 19, 180–186.

Kato, T.A., Shinfuku, N., Fujisawa, D., Tateno, M., Ishida, T., Akiyama, T., Sartorius, N., Teo, A.R., Choi, T.Y., Wand, A.P., Balhara, Y.P., Chang, J.P., Chang, R.Y., Shadloo, B., Ahmed, H.U., Lerthattasilp, T., Umene-Nakano, W., Horikawa, H., Matsumoto, R., Kuga, H., Tanaka, M., Kanba, S., 2011. Introducing the concept of modern depression in Japan; an international case vignette survey. Journal of Affective Disorders 135, 66–76.

Kijima, N., Saito, R., Takeuchi, M., Yoshino, A., Ono, Y., Kato, M., Kitamura, T., 1996. Cloninger's seven-factor model of temperament and character and Japanese version of temperament and character inventory (TCI). Archives of Psychiatric Diagnosis and Clinical Evaluation 7, 379–399, in Japanese.

Kijima, N., Tanaka, E., Suzuki, N., Higuchi, H., Kitamura, T., 2000. Reliability and validity of the Japanese version of the Temperament and Character Inventory. Psychological Reports 86, 1050–1058.

Kunugi, H., Urushibara, T., Nanko, S., 2004. Combined DEX/CRH test among Japanese patients with major depression. Journal of Psychiatric Research 38, 123–128.

Kunugi, H., Ida, I., Owashi, T., Kimura, M., Inoue, Y., Nakagawa, S., Yabana, T., Urushibara, T., Kanai, R., Aihara, M., Yuuki, N., Otsubo, T., Oshima, A., Kudo, K., Inoue, T., Kitaichi, Y., Shirakawa, O., Isogawa, K., Nagayama, H., Kamijima, K., Nanko, S., Kanba, S., Higuchi, T., Mikuni, M., 2006. Assessment of the dexamethasone/CRH test as a state-dependent marker for hypothalamic–pituitary–adrenal (HPA) axis abnormalities in major depressive episode: a Multicenter Study. Neuropsychopharmacology 31, 212–220.

Kunugi, H., Hori, H., Adachi, N., Numakawa, T., 2010. Interface between hypothalamic–pituitary–adrenal axis and brain-derived neurotrophic factor in depression. Psychiatry and Clinical Neurosciences 64, 447–459.

Künzel, H.E., Binder, E.B., Nickel, T., Ising, M., Fuchs, B., Majer, M., Pfennig, A., Ernst, G., Kern, N., Schmid, D.A., Uhr, M., Holsboer, F., Modell, S., 2003. Pharmacological and nonpharmacological factors influencing hypothalamic–pituitary–adrenocortical axis reactivity in acutely depressed psychiatric in-patients, measured by the Dex-CRH test. Neuropsychopharmacology 28, 2169–2178.

McCleery, J.M., Goodwin, G.M., 2001. High and low neuroticism predict different cortisol responses to the combined dexamethasone—CRH test. Biological Psychiatry 49, 410–415.

Mellsop, G.W., Hutton, J.D., Delahunt, J.W., 1985. Dexamethasone suppression test as a simple measure of stress? British Medical Journal (Clin. Res. Ed.) 290, 1804–1806.

Mendlowicz, M.V., Akiskal, H.S., Kelsoe, J.R., Rapaport, M.H., Jean-Louis, G., Gillin, J.C., 2005. Temperament in the clinical differentiation of depressed bipolar and unipolar major depressive patients. Journal of Affective Disorders 84, 219–223.

Miettunen, J., Veijola, J., Lauronen, E., Kantojärvi, L., Joukamaa, M., 2007. Sex differences in Cloninger's temperament dimensions–a meta-analysis. Comprehensive Psychiatry 48, 161–169.

Ministry of Health, Labour and Welfare, 2012. 〈http://www.mhlw.go.jp/kokoro/speciality/detail_depressive.html〉.

Nakano, K., 2005. Stress management, Kongo-syuppan, Tokyo. (in Japanese).

Nickel, T., Sonntag, A., Schill, J., Zobel, A.W., Ackl, N., Brunnauer, A., Murck, H., Ising, M., Yassouridis, A., Steiger, A., Zihl, J., Holsboer, F., 2003. Clinical and neurobiological effects of tianeptine and paroxetine in major depression. Journal of Clinical Psychopharmacology 23, 155–168.

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O'Leary, M.M., Taylor, J., Eckel, L., 2010. Psychopathic personality traits and cortisol response to stress: the role of sex, type of stressor, and menstrual phase. Hormones and Behavior 58, 250–256.

Oshima, A., Yamashita, S., Owashi, T., Murata, T., Tadokoro, C., Miyaoka, H., Kamijima, K., Higuchi, T., 2000. The differential ACTH responses to combined dexamethasone/CRH administration in major depressive and dysthymic disorders. Journal of Psychiatric Research 34, 325–328.

Ostergaard, S.D., Jensen, S.O., Bech, P., 2011. The heterogeneity of the depressive syndrome: when numbers get serious. Acta Psychiatrica Scandinavica 124, 495–496.

Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., Kamijima, K., 2005. Reliability and validity of Japanese version of the mini-international neuropsychiatric interview. Psychiatry and Clinical Neurosciences 59, 517–526.

Pancheri, P., Picardi, A., Pasquini, M., Gaetano, P., Biondi, M., 2002. Psychopathological dimensions of depression: a factor study of the 17-item Hamilton depression rating scale in unipolar depressed outpatients. Journal of Affective Disorders 68, 41–47.

Privitera, M.R., Greden, J.F., Gardner, R.W., Ritchie, J.C., Carroll, B.J., 1982. Interference by carbamazepine with the dexamethasone suppression test. Biological Psychiatry 17, 611–620.

Raison, C.L., Miller, A.H., 2003. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. American Journal of Psychiatry 160, 1554–1565.

Rinne, T., de Kloet, E.R., Wouters, L., Goekoop, J.G., DeRijk, R.H., van den Brink, W., 2002. Hyperresponsiveness of hypothalamic–pituitary–adrenal axis to combined dexamethasone/corticotropin-releasing hormone challenge in female borderline personality disorder subjects with a history of sustained childhood abuse. Biological Psychiatry 52, 1102–1112.

Rydmark, I., Wahlberg, K., Ghatan, P.H., Modell, S., Nygren, A., Ingvar, M., Asberg, M., Heilig, M., 2006. Neuroendocrine, cognitive and structural imaging characteristics of women on longterm sickleave with job stress-induced depression. Biological Psychiatry 60, 867–873.

Sasayama, D., Hori, H., Teraishi, T., Hattori, K., Ota, M., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Hashikura, M., Koga, N., Okamoto, N., Sakamoto, K., Higuchi, T., Amano, N., Kunugi, H., 2011. Difference in temperament and character inventory scores between depressed patients with bipolar II and unipolar major depressive disorders. Journal of Affective Disorders 132, 319–324.

Schüle, C., Baghai, T.C., Eser, D., Häfner, S., Born, C., Herrmann, S., Rupprecht, R., 2009. The combined dexamethasone/CRH Test (DEX/CRH test) and prediction of acute treatment response in major depression. PLoS One 4, e4324.

Schweitzer, I., Tuckwell, V., Maguire, K., Tiller, J., 2001. Personality pathology, depression and HPA axis functioning. Human Psychopharmacology 16, 303–308.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry 59 (suppl.20), 22–57.

Shimoda, M., 1957. Seishin eisei kowa, Dobunshoin, Tokyo. (in Japanese).

Spittlehouse, J.K., Pearson, J.F., Luty, S.E., Mulder, R.T., Carter, J.D., McKenzie, J.M., Joyce, P.R., 2010. Measures of temperament and character are differentially impacted on by depression severity. Journal of Affective Disorders 126, 140–146.

Svrakic, D.M., Whitehead, C., Przybeck, T.R., Cloninger, C.R., 1993. Differential diagnosis of personality disorders by the seven-factor model of temperament and character. Archives of General Psychiatry 50, 991–999.

Tarumi, S., Kanba, S., 2005. The dysthymic type of depression: an essay on sociocultural aspects of depression. Japanese Bulletin of Social Psychiatry 13, 129–136.

Tellenbach, H., 1961. Melancholie. Springer, Berlin.

Tops, M., Riese, H., Oldehinkel, A.J., Rijsdijk, F.V., Ormel, J., 2008. Rejection sensitivity relates to hypocortisolism and depressed mood state in young women. Psychoneuroendocrinology 33, 551–559.

Tyrka, A.R., Mello, A.F., Mello, M.F., Gagne, G.G., Grover, K.E., Anderson, G.M., Price, L.H., Carpenter, L.L., 2006. Temperament and hypothalamic–pituitary–adrenal axis function in healthy adults. Psychoneuroendocrinology 31, 1036–1045.

Tyrka, A.R., Wier, L.M., Price, L.H., Rikhye, K., Ross, N.S., Anderson, G.M., Wilkinson, C.W., Carpenter, L.L., 2008. Cortisol and ACTH responses to the Dex/CRH test: influence of temperament. Hormones and Behavior 53, 518–525.

Van Den Eede, F., Van den Bossche, B., Hulstijn, W., Sabbe, B.G., Cosyns, P., Claes, S.J., 2006. Combined dexamethasone/CRF test in remitted outpatients with recurrent major depressive disorder. Journal of Affective Disorders 93, 259–263.

Veen, G., Derijk, R.H., Giltay, E.J., van Vliet, I.M., van Pelt, J., Zitman, F.G., 2009. The influence of psychiatric comorbidity on the dexamethasone/CRH test in major depression. European Neuropsychopharmacology 19, 409–415.

Wahlberg, K., Ghatan, P.H., Modell, S., Nygren, A., Ingvar, M., Asberg, M., Heilig, M., 2009. Suppressed neuroendocrine stress response in depressed women on job-stress-related long-term sick leave: a stable marker potentially suggestive of preexisting vulnerability. Biological Psychiatry 65, 742–747.

Watson, S., Gallagher, P., Del-Estal, D., Hearn, A., Ferrier, I.N., Young, A.H., 2002. Hypothalamic–pituitary–adrenal axis function in patients with chronic depression. Psychological Medicine 32, 1021–1028.

Watson, S., Gallagher, P., Smith, M.S., Ferrier, I.N., Young, A.H., 2006. The dex/CRH test—is it better than the DST? Psychoneuroendocrinology 31, 889–894.

Wirtz, P.H., Siegrist, J., Schuhmacher, A., Hoefels, S., Maier, W., Zobel, A.W., 2010. Higher overcommitment to work is associated with higher plasma cortisol but not ACTH responses in the combined dexamethasone/CRH test in apparently healthy men and women. Psychoneuroendocrinology 35, 536–543.

Yehuda, R., Bierer, L.M., Schmeidler, J., Aferiat, D.H., Breslau, I., Dolan, S., 2000. Low cortisol and risk for PTSD in adult offspring of holocaust survivors. American Journal of Psychiatry 157, 1252–1259.

Zobel, A., Barkow, K., Schulze-Rauschenbach, S., Von Widdern, O., Metten, M., Pfeiffer, U., Schnell, S., Wagner, M., Maier, W., 2004. High neuroticism and depressive temperament are associated with dysfunctional regulation of the hypothalamic–pituitary–adrenocortical system in healthy volunteers. Acta Psychiatrica Scandinavica 109, 392–399.

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〔特集：うつ病の最先端脳科学的研究とその臨床応用〕

視床下部-下垂体-副腎系とうつ病：最近の展開*

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要約：うつ病はストレスが誘因となることが多く，ストレス応答で重要な役割を果たす視床下部-下垂体-副腎系（hypothalamic-pituitary-adrenal axis：HPA 系）は，うつ病の病態において重要な役割を果たすことはさまざまな点から裏付けられている．筆者らは，1990 年代に開発された DEX/CRH テストを用いて，うつ病患者の HPA 系を継続的に検討し，同テストの感度が高く，状態依存性マーカーとして有用であることを報告した．すなわち，うつ病入院患者では治療前に HPA 系が機能亢進しているが，治療によって回復すると HPA 系も正常に近づくことを示した．この結果に基づいて，筆者らは，過剰なグルココルチコイドの細胞障害作用について，脳由来神経栄養因子（BDNF）の機能に焦点を当てて細胞生物学的に検討した．その結果，合成グルココルチコイドであるデキサメサゾンは，発達期の海馬ニューロンの BDNF に誘起される突起伸長やシナプス形成を阻害すること，大脳皮質ニューロンにおいて，グルココルチコイド受容体は BDNF の特異的受容体 TrkB と相互作用しており，グルココルチコイドは BDNF の急性作用であるグルタミン酸放出作用を阻害することを見いだした．以上の結果は，グルココルチコイドの新しい細胞障害作用メカニズムとして注目される．MRI を用いたマクロの視点では，DEX/CRH テストでグルココルチコイドの過剰を示す者（健常女性）は，いくつかの脳領域で脳皮質体積の減少や拡散テンソル画像でみられる神経ネットワークの低下がみられた．しかし，近年，外来での軽症うつ病や慢性うつ病などの検討では，HPA 系の過剰がみられないという報告が増えている．筆者らは，一般人口の約 1 割に HPA 系の過剰抑制ないしコルチゾール低下（hypocortisolism）を示す者が存在することを見いだした．過剰抑制を示す健常者の心理特性について検討したところ，ディストレス（ストレス症状）が強いこと，非適応的なコーピング様式，統合失調症型人格傾向などを示すことを見いだした．以上の結果は，HPA 系の過剰抑制もうつ病スペクトラムにおいて重要であることを示唆し，今後はこの点に焦点を当てていく必要があると考えられる．

キーワード：うつ病，脳由来神経栄養因子（BDNF），視床下部-下垂体-副腎系，コルチゾール低下，磁気共鳴画像（MRI）

視床下部-下垂体-副腎系（hypothalamic-pituitary-adrenal axis: HPA 系）は自律神経系や免疫系とともにストレス応答において中心的役割を果たしている．うつ病はストレスによって誘発される代表的な精神疾患であり，HPA 系とうつ病との関係は古くから検討がなされている．しかし，うつ病には異種性があることから，うつ病が HPA 系の負のフィードバックの低下によって生じるという図式が必ずしも当てはまらないことが明らかになっている．また，ミクロでみた HPA 系のニューロンへの影響に関する知見や，マクロでみた HPA 系の脳形態への影響に関する知見も蓄積されてきている．

筆者らも 10 年以上にわたり，うつ病における HPA 系の役割について検討してきた．そこで，筆者らの検討を中心に，I. うつ病とストレスホルモン（グルココルチコイド）との関係―DEX/CRH テストを中心に，II. ミクロでのグルココルチコイドの影響―BDNF 機能への影響に注目して，III. マクロでのグルココルチコイドの影響―MRI 研究，IV. ストレスホルモン不足とうつ病スペクトラム，についての成果を紹介する．

I. うつ病とストレスホルモン（グルココルチコイド）―DEX/CRH テストを中心に―

典型的なメランコリー型の大うつ病の診断基準を満たす者には，HPA 系の機能亢進を示す者が多いことは古くから指摘されている．コルチゾールの過剰分泌を示す所見として尿中遊離コルチゾール値の上昇，血液や脳脊髄液レベルの上昇，コルチゾール分泌概日リズムの異常（日内変動の振幅の低下），デキサメサゾン抑制試験（DST）におけるコルチゾールの非抑制によって検出される HPA 系の負のフィードバック機構の低下，脳脊髄液中の CRH 高値，リンパ球のグルココルチコイド受容体数の減少，CRH 負荷テストで ACTH 反応が低下，自殺者死後脳での CRH 受容体結合数の減少，副腎肥大や下垂体肥大などが報告されており，多くの面から裏付けられている．

以上のような種々の異常のうち，日常臨床での診断に応用が期待されたのは，DST で非抑制を呈することである．気分障害を対象とした DST のプロトコールは種々の方法があるが，通常は，前夜 23 時にデキサメサゾン 0.5 mg ないし 1 mg を経口投与し，検査当日 1～3 回時刻を変えて採血を行い（最も簡便に行う場合，16 時に 1 回採血），血漿コルチゾールを測定する（できれば ACTH も測定）．健

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常者では，デキサメサゾンによってコルチゾールやACTHの分泌は約24時間抑制されるが，翌日のコルチゾール値が5 μg/dl以上を呈した場合は非抑制と判定する．DSTは当初，内因性うつ病の診断に有用である可能性が期待され（Carroll, 1982），1980年代を中心に精力的に検討された．しかし，米国精神医学会による大うつ病数千例の検討によれば，デキサメサゾン1 mgを用いて測定した場合の感受性（患者の中でDSTが非抑制となる者の割合）はおよそ40～50%であるとされ，健常者における特異性（非患者の中でDSTが抑制となる割合）はおよそ90%であったと結論している（American Psychiatric Association, 1987）．日本での検討も多数あり，やはり感受性が高くないという問題が指摘されていた．

上記のようにDSTは感受性が概して高くないことや，うつ病以外の他の精神疾患においても非抑制例が少なくないこともあり，初期の検討では診断に用いることには無理があるという結論に至っている．しかし，DSTにおいて非抑制型であった者も治療によって回復した後に再度DSTを施行すると抑制型に転じることや，再発予後や自殺行動の予測因子となる可能性なども指摘され，少なくとも一部の患者では治療経過の指標として臨床的に有用である可能性があり，研究レベルでの検討は続いている．

その後，ドイツのマックスプランク研究所のHolsboerらのグループは，DSTとCRH負荷テストを組み合わせた「DEX/CRHテスト」（図1）を開発し（Heuser et al, 1994），従来のDSTに比べて感度が高いことを報告している（～80%）．筆者らによる日本人の大うつ病（入院患者）と健常者との比較でも，DEX/CRH負荷後血漿コルチゾール値が5 μg/dl以上を呈した患者はおよそ70%に達し，感度が高いことが支持された（Kunugi et al, 2004）．その後，筆者らも参加した日本の多施設研究による入院患者のデータでは，入院時にDEX/CRHテストでコルチゾールが高値を示し，治療によってうつ病が改善するとHPA系が正常に近づくことが明らかになった（図2）．特に，抗うつ薬による薬物療法に加えて通電療法を行った群では，大きな変動（反応の正常化）がみられた．また，入院時に既に抗うつ薬を投与されていたか否かと，HPA系の反応とは関連がなかった．以上から，DEX/CRHテストによって検出されるHPA系の異常は，抗うつ薬による直接的影響によるものではなく，うつ病の回復によって正常化する状態依存的指標（state-dependent marker）の特徴をもつことが示された．DEX/CRHテストは従来のDSTと比較すると特異性はむしろ下がるが，DSTより感度が高いことから，より多数のうつ病例のモニターに有用である可能性がある．

筆者らは，健常者のDEX/CRHテスト結果に関しても種々の精神症状・生理学的指標との関連を検討している．その結果，DEX/CRHテストで非抑制を呈しHPA系が亢進している者は，ピッツバーグ睡眠質問票で評価した場合，睡眠の質が低下していることが明らかになった（Hori et al, 2011b）．また，健常者でDEX/CRHテストでHPA系の過剰抑制を示す群は，種々のストレス症状や不適応的なコーピング，スキゾイド傾向などを示すことが明らかになったが，これについてはIVで詳しく述べる．

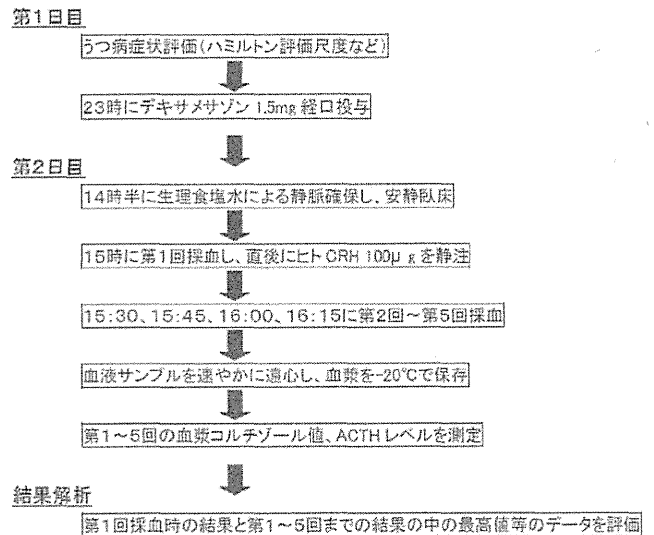

図1　DEX/CRHテストのプロトコール．結果の評価方法は，いまだに議論のあるところであるが，筆者らは，第1回のコルチゾール値が5 μg以上であれば非抑制，第1回のコルチゾール値が5 μg未満で，最高値が5 μg以上であれば中間抑制，最高値が5 μg未満なら抑制，最高値が測定感度（1 μg）未満であれば過剰抑制と，4段階評価を行っている．非抑制と中間抑制とを非抑制としてまとめる場合もある．近年，筆者らは，簡便法として，第2日目の採血を15時と16時の2回のみ行い，ACTHの測定を行わずにコルチゾールのみを測定する方法を用いている．

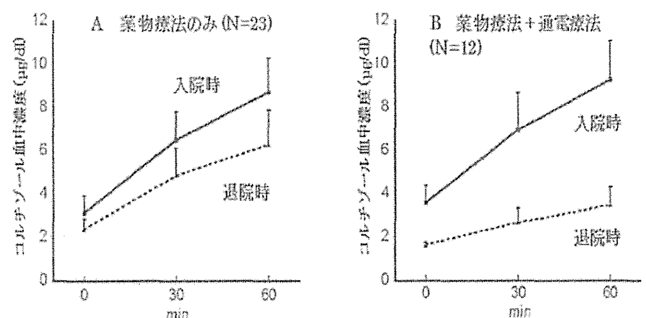

図2　うつ病患者における治療前後でのDEX/CRHテスト所見の変化．A. 抗うつ薬治療のみ，B. 抗うつ薬と通電療法の併用．エラーバーは標準誤差を示す．（Kunugi et al, 2006を改変）

II.　ミクロでのグルココルチコイドの影響
—BDNF機能への影響に注目して—

それでは，ストレスや過剰なグルココルチコイドがうつ病を惹起するメカニズムはどのようなものであろうか．その1つとして，脳由来神経栄養因子（brain-derived

neurotrophic factor: BDNF）を介した仮説が注目されている（Duman and Monteggia, 2006）．種々の動物実験によって，ストレス（拘束，予測不能，電気ショック，社会的孤立，社会的敗北，母子分離など）やグルココルチコイド投与が海馬でのBDNF発現を低下させることが報告されている．BDNFは，神経の成長・分化・生存を促進することから，発現の低下によって海馬が障害され，HPA系の制御やニューロン新生などの低下につながる可能性がある．

筆者らは，グルココルチコイドのニューロンへの影響についてBDNFの発現ではなく，BDNFの受容体を介した機能への影響について細胞生物学的に検討した．

1. グルココルチコイドは海馬ニューロンにおけるBDNFの長期的なシナプス形成作用を阻害する

筆者らは，生後1日齢のラットより分散培養した海馬ニューロンにおいて，デキサメサゾンで処理しておくと，2日後に観察されたBDNFによる突起の伸展促進効果やシナプス形成効果が阻害されることを観察した（Kumamaru et al, 2008）．興味深いことに，初期にデキサメサゾンに暴露すると16日後のニューロンにおけるシナプス数も抑制されていたことから，幼若期におけるデキサメサゾン投与の影響は，成熟後のニューロンネットワーク全体の機能低下をもたらす可能性が示唆された．この阻害機能に関与する細胞内シグナルとしては，mitogen-activated protein kinase（MAPK）経路が重要であることを見いだした．

2. グルココルチコイドは大脳皮質ニューロンにおけるBDNFの短期的な神経伝達物質放出作用も阻害する

BDNFには，1. のような遺伝子/蛋白質の発現を介すると考えられる長期的な機能に加えて，遺伝子発現を介さない非常に短時間での機能もある．なかでも，神経伝達物質の放出を誘導する機能は重要である．筆者らはこれまでにBDNFが大脳皮質ニューロンから急速に興奮性神経伝達物質であるグルタミン酸の放出を引き起こす現象を見いだし，それにはPhospholipase C γ（PLC-γ）経路で誘導される細胞内Ca^{2+}ストアからのCa^{2+}放出が重要であることなどを報告してきた（Matsumoto et al, 2006）．そこで，このBDNFの作用に対するグルココルチコイドの効果を詳細に解析したところ，驚いたことにTrkBとグルココルチコイド受容体（GR）が相互作用することを発見し，この相互作用がPLC-γ/Ca^{2+}シグナルを制御することを明らかにした（Numakawa et al, 2009）．

大脳皮質ニューロンに48時間のデキサメサゾン暴露を行い，BDNF投与1分後の細胞応答をみると，デキサメサゾン処理群ではグルタミン酸放出が減少し，BDNFによる細胞内Ca^{2+}増加を抑制した．注目すべきことに，デキサメサゾン投与後，GRの発現低下が観察された．またGRとBDNFの特異的受容体TrkBとの相互作用もそれに応じて減少した．デキサメサゾンを腹腔投与したラット大脳組織のスライス培養でもGR発現とそのTrkBとの相互作用の低下がみられたことから，この相互作用が生理的条件下でも重要な役割を果たしている可能性が示唆された．また，siRNAによってGR発現を抑制したニューロンでは，BDNFによるグルタミン酸放出が低下することを筆者らは見いだした．逆に，GRの強制発現を行うと，グルタミン酸放出が増加した．これらの結果は，GRの発現そのものが重要であり，転写因子としての作用は関与しない可能性を示唆する．さらに，デキサメサゾンによって，TrkB下流シグナルの中で特にPLC-γ/Ca^{2+}経路が抑制されていたことから，この経路への影響を介してグルタミン酸放出が阻害されることが示唆された．なお，この実験系ではデキサメサゾン暴露による内在性のBDNFやTrkBの発現変化は観察されなかった．

以上から，これまで多くの研究者が転写因子として認識していたGRが，膜蛋白であるTrkBとの相互作用を介してBDNFの働きを制御している，というGRの新しい機能が明らかになった．この結果から，ストレスホルモンへの過剰な暴露によるBDNF機能低下について図3のようなスキーマが考えられる．

III. マクロでのグルココルチコイドの影響―MRI研究―

それでは，上記のようなミクロな変化はMRI脳画像などのマクロの視点でとらえることができるだろうか？ これまでに，血漿コルチゾールや24時間尿中フリーコルチゾール値がMRIで測定した海馬体積と逆相関するという報告や（Lupien et al, 1998; Wolf et al, 2002），DEX/CRHテストでHPA系の機能亢進を示した者は前頭葉が萎縮している傾向があるという報告などがあるが（Gold et al, 2005），関心領域（region of interest: ROI）を設定せずに検討した研究や，拡散テンソル画像を用いた検討はほとんどなく，グルココルチコイドによる脳へのマクロの影響についての検討は不十分であった．

筆者らは，健常者女性（N=34）を対象として，DEX/CRHテスト所見とMRI脳画像による脳構造体積，拡散テンソル画像との関連について関心脳領域を設定しない包括的な検討を行った．その結果，脳構造体積では，側頭葉や後頭葉の一部に皮質体積の減少を認め，拡散テンソル画像では，脳の広範な領域において平均拡散係数（mean diffusivity）の低下が認められ，神経ネットワークの低下があることが明らかになった（Otaら，未発表）．この神経ネットワークの低下は，特に，前部帯状回，後頭領域，側頭極，脳梁吻側において強かった．

以上のように，DEX/CRHテストでとらえることのできるストレスホルモンの過剰は，健常者においてもマクロのレベルでとらえることのできる脳の構造や神経線維ネットワークに障害を与えている．

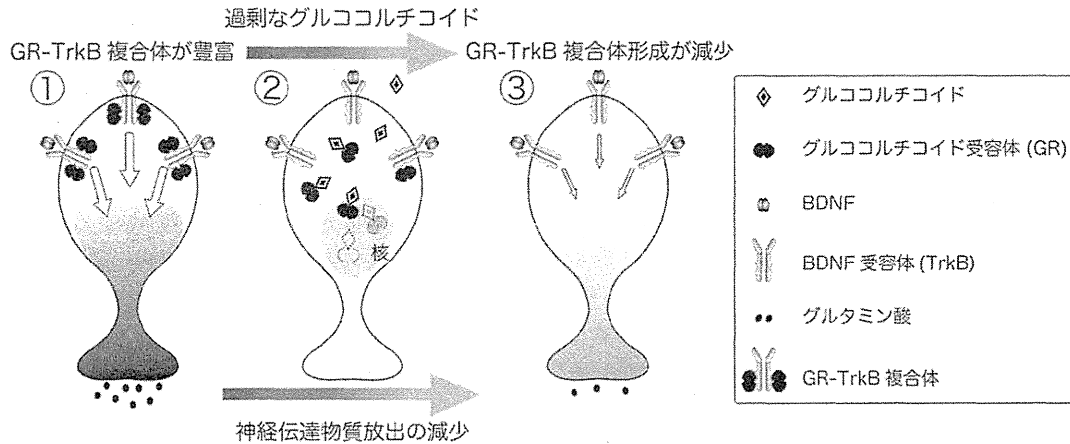

図3 グルココルチコイド暴露後におけるBDNFの短期的作用.（神経伝達物質放出能力の抑制）（沼川, 2009より）
①グルココルチコイド受容体（GR)-TrkB 複合体が豊富であり, BDNFによるPLC活性化（グルタミン酸放出に必要なシグナル分子）が十分でグルタミン酸も多く放出. ②グルココルチコイド暴露でGRが減少し, GR-TrkB 複合体の割合が低下. ③GR-TrkB 複合体量の低下により, PLC活性化が不十分でグルタミン酸放出が減少.

IV. ストレスホルモン不足とうつ病スペクトラム

DEX/CRH テストやDSTを用いた, より最近の研究においては, うつ病においても, 平均するとHPA系の亢進を認めないという報告もなされるようになり, うつ病患者のコルチゾール値は健常者と変わらないという報告や(Carpenter et al, 2009; Gervasoni et al, 2004; Watson et al, 2002), むしろコルチゾール低下 (hypocortisolism) であるという報告さえなされている (Levitan et al, 2002; Rydmark et al, 2006). そうしたうつ病患者の特徴として, 外来患者, 慢性患者, 長期病気休暇中の患者, 非定型うつ病などが挙げられている. また, 慢性の経過をたどることの多い疾患である心的外傷後ストレス障害 (PTSD) では, DSTに対してむしろ過剰抑制を示すことや (Yehuda et al, 1993), 外傷となるイベント発生後の期間が長くなるにつれて過剰抑制がさらに顕著になることが知られている (Yehuda et al, 2004). 慢性疲労症候群 (Gaab et al, 2002) や線維筋痛症 (Wingenfeld et al, 2007) でもコルチゾール低値の傾向にある. さらに, アジソン病 (慢性副腎機能低下症) やACTH単独欠損症のようにコルチゾールが低下している病気では, 全身倦怠感, 食欲低下, 体重減少, 無気力, 不安などのうつ病とよく似た症状を呈することはよく知られている. これらをまとめると, 非抑制のみならず, 過剰抑制もうつ病症状をきたすHPA系機能異常として重要であると考えられる. したがって, うつ病におけるHPA系異常の知見が一致していない背景に, うつ病の異種性 (例えば, メランコリー型うつ病 vs. 非定型うつ病) やHPA系の過剰抑制がある可能性が高い.

筆者らは, これまで10年以上にわたりおよそ500名の被験者に対して, DEX/CRHテストを施行し, データを蓄積してきた. その中で, 人口のおよそ1割程度に, HPA系が過剰に抑制されている群が存在することを見いだした (図4). すなわち, DEX/CRHテストにおいてCRH刺激の1時間後にもグルココルチコイドが測定感度 (1 μg/dl) 未満に抑制されている群である. そこで, このような過剰抑制を示す健常者のディストレス (distress) やコーピング, パーソナリティ特性などについて検討を行った.

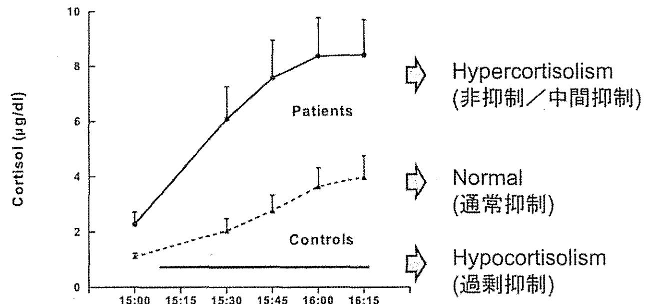

図4 DEX/CRH test による過剰抑制者の検出. 健常者のおよそ10% はコルチゾール反応が非常に低い (CRH負荷1時間後にもコルチゾール濃度<1.0 μg/dl).

ディストレスとは, ストレスをうまく処理できないことによって生じる心身の不調のことであり,「心理的苦痛」などと訳される. コーピングとは, ストレスのかかる状況に対処するために用いる, 思考ないし行動のことである. ディストレスやコーピングとHPA系機能との関連を検討した研究はいまだに少ない.

筆者らは, Hopkins Symptom Checklist (HSCL) を用い, テスト前1週間のディストレス (ストレス関連症状) の程度を調べた. HSCLは54項目からなる自記式質問紙であり, 5つの症状尺度 (心身症状, 強迫症状, 対人関係過敏症状, 不安症状, 抑うつ症状) について評価する. コーピング様式は, Ways of Coping Checklist (WCCL) を用いて評価した. WCCLは47項目からなる自記式質問紙であり,

6次元のコーピング様式（問題解決，積極的認知対処，ソーシャルサポート，自責，希望的観測，回避）についてスコアリングする．問題解決と積極的認知対処は「問題焦点型」とされ，ソーシャルサポートとともに適応的なコーピング様式とされ，自責や希望的観測，回避は「情動焦点型」と呼ばれ，一般に不適応的でストレスの解決になりにくいコーピング様式である．

ディストレスとDEX/CRHテスト抑制パターンの関連についての結果を図5に示した（Hori et al, 2010）．過剰抑制群では，他の2群（通常抑制群と非抑制）に比べ，強迫症状，対人関係過敏症状，不安症状が有意に強かった．コーピングとDEX/CRHテスト抑制パターンの関連についての結果を図6に示した．過剰抑制群では，他の2群に比べて回避のコーピングを用いる傾向が有意に強いことが示された．有意差はみられなかったものの，過剰抑制群では，自責・希望的観測といった他の情動焦点型コーピング様式の得点も高かった．

これらの結果から，HPA系の過剰抑制は強迫や対人過敏，不安などのディストレスや情動焦点型コーピングと関連することが示唆された．非抑制はこれらの心理特性と関連しなかった．先行研究でも，コルチゾール低値が拒絶に対する過敏性や受動的コーピングと関連することが報告されている（Tops et al, 2008）．ただし，コルチゾール高値が否認や受動性に関連するという報告もあり，知見は必ずしも一致していない．

続いて，筆者らは141名の健常成人にDEX/CRHテストを施行し，HPA系機能とパーソナリティとの関連について検討を行った（Hori et al, 2011a）．クローニンジャーのTemperament and Character Inventory（TCI）で評価した気質・性格次元に加え，Schizotypal Personality Questionnaire（SPQ）で測定した統合失調型パーソナリティ傾向と，DEX/CRHテストに対するコルチゾールの反応性との関連を調べた．TCIは240項目からなる自記式質問紙であり，4つの気質次元（新奇性追求／損害回避／報酬依存／持続）と3つの性格次元（自己志向／協調性／自己超越性）について評価する．SPQは74項目からなる自記式質問紙であり，9つの下位尺度（関係念慮／不適切な，または限定された感情／奇異な信念／奇異な行動または外見

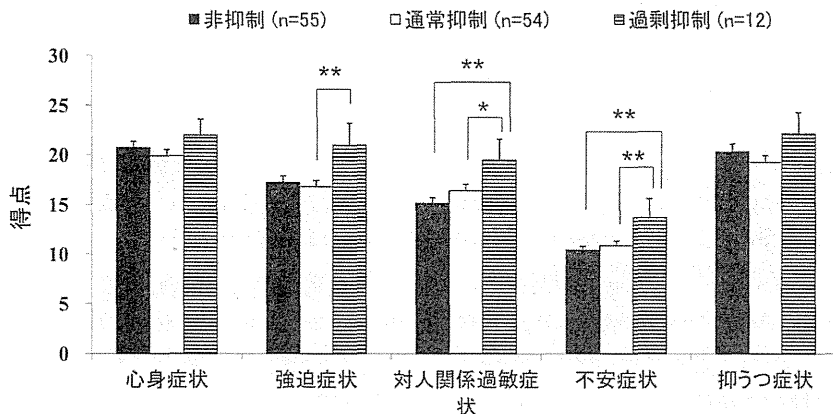

図5 ディストレスとDEX/CRHテスト抑制パターン．（Hori et al, 2010を改変）*p＜0.05; **p＜0.01（共分散分析による，性別と年齢を統制した推定周辺平均に基づいたペアごとの比較．多重比較についてはBonferroniによる補正を行った）．エラーバーは標準誤差を示す．

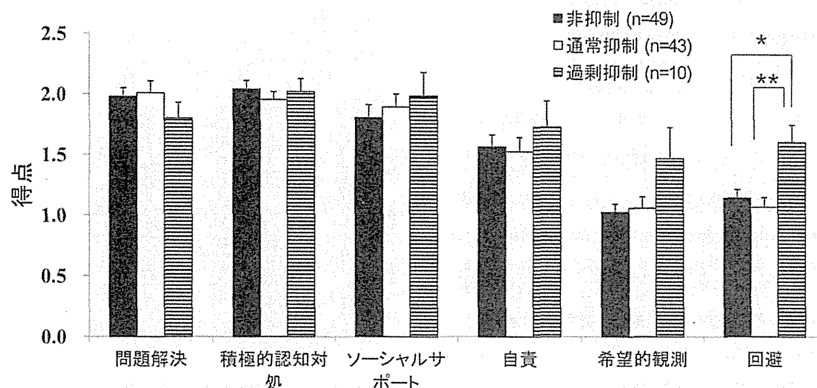

図6 コーピング特性とDEX/CRHテスト抑制パターン．（Hori et al, 2010を改変）*p＜0.05; **p＜0.01（共分散分析による，性別と年齢を統制した推定周辺平均に基づいたペアごとの比較．多重比較についてはBonferroniによる補正を行った）．エラーバーは標準誤差を示す．

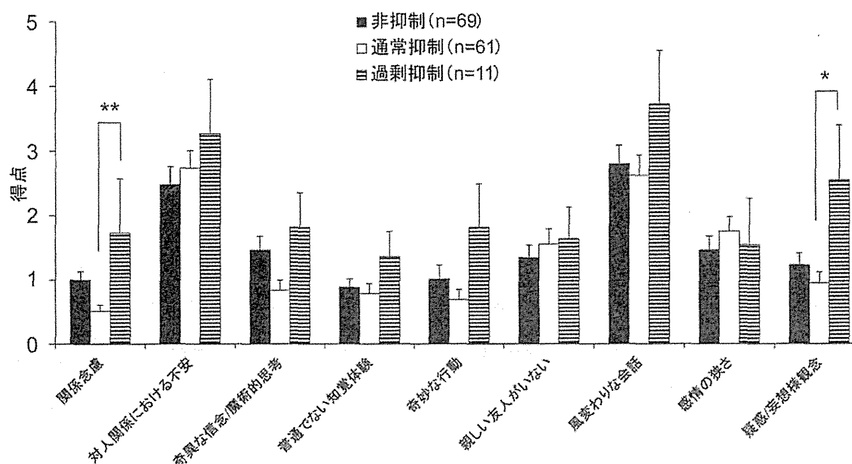

図7　統合失調型パーソナリティと DEX/CRH テスト抑制パターン.（Hori et al, 2011a を改変）$^{*}p<0.05$; $^{**}p<0.01$（共分散分析による，性別と年齢を統制した推定周辺平均に基づいたペアごとの比較．多重比較については Bonferroni による補正を行った）エラーバーは標準誤差を示す.

／普通でない知覚体験／親しい友人がいない／奇異な考え方・話し方／過剰な社会不安／疑惑・妄想様観念）と，それらの合計得点である.

SPQ の下位項目と DEX/CRH テスト結果との関連を図7に示す．それによると，関係念慮や疑惑・妄想様観念という統合失調症の陽性症状に対応する項目と HPA 系の過剰抑制が有意に関連していた．一方，TCI の7次元については，いずれも DEX/CRH テスト結果とは有意な関連を認めなかった.

今回の筆者らの結果は，コルチゾール低値（あるいは過剰抑制）と関連することが知られている非定型うつ病の特徴の1つが対人過敏性や回避である，という事実に合致する．また，回避のコーピングやシゾイド傾向との関連から，逃避型うつ病（広瀬）やシゾイドうつ病（牛島）などとも関連するかもしれない．しかし，これについては今のところデータがなく，今後，検討する価値がある.

表　HPA 系の過剰抑制（ストレスホルモン不足）と関連する病態

- 慢性疲労症候群
- 線維筋痛症
- 心的外傷後ストレス障害
- 非定型うつ病
- 逃避型うつ病？
- シゾイドうつ病？
- アジソン病
- ACTH 欠損症

V. おわりに

視床下部-下垂体-副腎系とうつ病との関連について，筆者らの最近の検討を中心に述べた．HPA 系を検出する上で比較的感度の良い DEX/CRH テストは状態依存性マーカーの特徴をもち，非抑制を示す者については経過の指標として有用である可能性が高い．しかし，古典的な HPA 系の負のフィードバックの低下（すなわち HPA 系の亢進）を示す群は，入院患者のような典型的なメランコリー型の特徴であって，外来などでみられる多くの“うつ病”に当てはまらないことも明らかである．近年の外来うつ病の検討では，HPA 系が亢進している者から過剰に抑制されている者まで存在し，それぞれが異なった病態をもっている可能性がある．筆者らは，HPA 系の過剰抑制を示す一連のうつ病スペクトラムが存在すると考えている（表）．現在，うつ病の中で非抑制を示す群と過剰抑制を示す群との臨床特性の違いについて詳細な検討を行っている.

HPA 系がどのように脳機能を障害するか，ミクロとマクロのメカニズムについて，筆者らの検討を紹介した．ミクロの視点では，今後，BDNF 以外の神経栄養因子の関与や，グルココルチコイドが欠乏した場合の変化について検討していきたい．また，脳画像研究では HPA 系の過剰抑制の影響などについて検討していきたいと考えている.

文　献

American Psychiatric Association (1987) The dexamethasone suppression test: An overview of its current status in psychiatry. The APA task force on laboratory tests in psychiatry. Am J Psychiatry, 144: 1253-1262.

Carpenter, L. L., Ross, N. S., Tyrka, A. R., Anderson, G. M., Kelly, M. and Price, L. H. (2009) Dex/CRH test cortisol response in outpatients with major depression and matched healthy controls. Psychoneuroendocrinology, 34: 1208-1213.

Carroll, B. J. (1982) The dexamethasone suppression test for melancholia. Br J Psychiatry, 140: 292-304.

Duman, R. S. and Monteggia, L. M. (2006) A neurotrophic model for stress-related mood disorders. Biol Psychiatry, 59: 1116-1127.

Gaab, J., Hüster, D., Peisen, R., Engert, V., Schad, T., Schürmeyer, T.H. and Ehlert, U. (2002) Low-dose dexamethasone suppression test in chronic fatigue syndrome and health. Psychosom Med, 64: 311-318.

Gervasoni, N., Bertschy, G., Osiek, C., Perret, G., Denis, R., Golaz, J., Rossier, M. F., Bondolfi, G. and Aubry, J. M. (2004) Cortisol responses to combined dexamethasone/CRH test in outpatients with a major depressive episode. J Psychiatr Res, 38: 553-557.

Gold, S. M., Dziobek, I., Rogers, K., Bayoumy, A., McHugh, P. F. and Convit, A. (2005) Hypertension and hypothalamo-pituitary-adrenal axis hyperactivity affect frontal lobe integrity. J Clin Endocrinol Metab, 90: 3262-3267.

Heuser, I., Yassouridis, A. and Holsboer, F. (1994) The combined dexamethasone/CRH test: A refined laboratory test for psychiatric disorders. J Psychiatr Res, 28: 341-356.

Hori, H., Ozeki, Y., Teraishi, T., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Suto, S., Terada, S., Higuchi, T. and Kunugi, H. (2010) Relationships between psychological distress, coping styles, and HPA axis reactivity in healthy adults. J Psychiatr Res, 44: 865-873.

Hori, H., Teraishi, T., Ozeki, Y., Hattori, K., Sasayama, D., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Higuchi, T. and Kunugi, H. (2011a) Schizotypal personality in healthy adults is related to blunted cortisol responses to the combined dexamethasone/corticotropin-releasing hormone test. Neuropsychobiology, 63: 232-241.

Hori, H., Teraishi, T., Sasayama, D., Ozeki, Y., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Hattori, K., Higuchi, T. and Kunugi, H. (2011b) Poor sleep is associated with exaggerated cortisol response to the combined dexamethasone/CRH test in a non-clinical population. J Psychiatr Res, 45: 1257-1263.

Kumamaru, E., Numakawa, T., Adachi, N., Yagasaki, Y., Izumi, A., Niyaz, M., Kudo, M. and Kunugi, H. (2008) Glucocorticoid prevents brain-derived neurotrophic factor-mediated maturation of synaptic function in developing hippocampal neurons through reduction in the activity of mitogen-activated protein kinase. Mol Endocrinol, 22: 546-558.

Kunugi, H., Urushibara, T. and Nanko, S. (2004) Combined DEX/CRH test among Japanese patients with major depression. J Psychiatr Res, 38: 123-128.

Kunugi, H., Ida, I., Owashi, T., Kimura, M., Inoue, Y., Nakagawa, S., Yabana, T., Urushibara, T., Kanai, R., Aihara, M., Yuuki, N., Otsubo, T., Oshima, A., Kudo, K., Inoue, T., Kitaichi, Y., Shirakawa, O., Isogawa, K., Nagayama, H., Kamijima, K., Nanko, S., Kanba, S., Higuchi, T. and Mikuni, M. (2006) Assessment of the dexamethasone/CRH test as a state-dependent marker for hypothalamic-pituitary-adrenal (HPA) axis abnormalities in major depressive episode: A multicenter study. Neuropsychopharmacology, 31: 212-220.

Levitan, R. D., Vaccarino, F. J., Brown, G. M. and Kennedy, S. H. (2002) Low-dose dexamethasone challenge in women with atypical major depression: Pilot study. J Psychiatry Neurosci, 27: 47-51.

Lupien, S. J., de Leon, M., De Santi, S., Convit, A., Tarshish, C., Thakur, M., McEwen, B. S., Hauger, R. L. and Meaney, M. J. (1998) Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat Neurosci, 1: 69-73.

Matsumoto, T., Numakawa, T., Yokomaku, D., Adachi, N., Yamagishi, S., Numakawa, Y., Kunugi, H. and Taguchi, T. (2006) Brain-derived neurotrophic factor-induced potentiation of glutamate and GABA release: Different dependency on signaling pathways and neuronal activity. Mol Cell Neurosci, 31: 70-84.

沼川忠広 (2009) グルココルチコイド受容体およびBDNF受容体の相互作用に制御されるグルタミン酸放出. 神経化学, 48: 301-307.

Numakawa, T., Kumamaru, E., Adachi, N., Yagasaki, Y., Izumi, A. and Kunugi, H. (2009) Glucocorticoid receptor interaction with TrkB promotes BDNF-triggered PLC-gamma signaling for glutamate release via a glutamate transporter. Proc Natl Acad Sci USA, 106: 647-652.

Rydmark, I., Wahlberg, K., Ghatan, P.H., Modell, S., Nygren, A., Ingvar, M., Asberg, M. and Heilig, M. (2006) Neuroendocrine, cognitive and structural imaging characteristics of women on longterm sickleave with job stress-induced depression. Biol Psychiatry, 60: 867-873.

Tops, M., Riese, H., Oldehinkel, A. J., Rijsdijk, F. V. and Ormel, J. (2008) Rejection sensitivity relates to hypocortisolism and depressed mood state in young women. Psychoneuroendocrinology, 33: 551-559.

Watson, S., Gallagher, P., Del-Estal, D., Hearn, A., Ferrier, I. N. and Young, A. H. (2002) Hypothalamic-pituitary-adrenal axis function in patients with chronic depression. Psychol Med, 32: 1021-1028.

Wingenfeld, K., Wagner, D., Schmidt, I., Meinlschmidt, G., Hellhammer, D. H. and Heim, C. (2007) The low-dose dexamethasone suppression test in fibromyalgia. J Psychosom Res, 62: 85-91.

Wolf, O. T., Convit, A., de Leon, M. J., Caraos, C. and Qadri, S. F. (2002) Basal hypothalamo-pituitary-adrenal axis activity and corticotropin feedback in young and older men: Relationships to magnetic resonance imaging-derived hippocampus and cingulate gyrus volumes. Neuroendocrinology, 75: 241-249.

Yehuda, R., Southwick, S. M., Krystal, J. H., Bremner, D., Charney, D. S. and Mason, J. W. (1993) Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. Am J Psychiatry, 150: 83-86.

Yehuda, R., Halligan, S. L., Golier, J. A., Grossman, R. and Bierer, L. M. (2004) Effects of trauma exposure on the cortisol response to dexamethasone administration in PTSD and major depressive disorder. Psychoneuroendocrinology, 29: 389-404.

Abstract: Hiroshi KUNUGI, Hiroaki HORI, Tadahiro NUMAKAWA and Miho OTA (Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502 Japan) *The hypothalamic-pituitary-adrenal axis and depressive disorder: Recent progress*. Jpn. J. Neuropsychopharmacol., 32: 203-209 (2012).

Depression is a stress-induced disorder and there is compelling evidence for the involvement of hypothalamic-pituitary-adrenal (HPA) axis abnormalities in the disease. Chronic hyperactivity of the HPA axis and resultant excessive glucocorticoid (hypercortisolism) may be causal to depression. We demonstrated that the dexamethasone (DEX)/CRH test is a sensitive state-dependent marker to monitor HPA axis abnormalities. Restoration from HPA axis abnormalities occurs with clinical responses to treatment. Brain-derived neurotrophic factor (BDNF) has also been implicated in depression. We found that glucocorticoid (DEX) suppresses BDNF-induced dendrite outgrowth and synaptic formation via blocking the MAPK pathway in early-developing cultured hippocampal neurons. Furthermore, we demonstrated that glucocorticoid receptor (GR) and TrkB (a specific receptor of BDNF) interact and that DEX acutely suppresses BDNF-induced glutamate release by affecting the PLC-γ pathway in cultured cortical neurons, indicating a mechanism underlying the effect of excessive glucocorticoid on BDNF function and resultant damage in cortical neurons. In a macroscopic view using magnetic resonance imaging (MRI), we found that individuals with hypercortisolism detected by the DEX/CRH test demonstrated volume loss in gray matter and reduced neural network assessed with diffusion tensor imaging in several brain regions. Finally, we observed that individuals with hypocortisolism detected by the DEX/CRH test tend to present more distress symptoms, maladaptive coping styles, and schizotypal personality traits than their counterparts, which points to the important role of hypocortisolism as well as hypercortisolism in depression spectrum disorders.

Key words: Depression, Brain-derived neurotrophic factor (BDNF), Hypothalamic-pituitary-adrenal axis (HPA axis), Hypocoltisolism, Magnetic resonance imaging (MRI)

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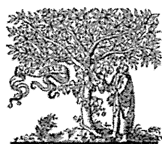

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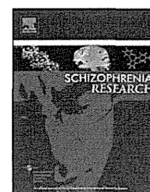

Negative correlation between cerebrospinal fluid oxytocin levels and negative symptoms of male patients with schizophrenia

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ABSTRACT

Background: Accumulating evidence indicates that oxytocin plays an important role in social interactions. Previous studies also suggest altered oxytocin function in patients with schizophrenia and depression. However, few studies have examined the central oxytocin levels in these disorders.
Methods: Cerebrospinal fluid (CSF) oxytocin levels were measured by ELISA in male participants consisting of 27 patients with schizophrenia, 17 with major depressive disorder (MDD), and 21 healthy controls.
Results: CSF oxytocin levels of patients with schizophrenia or MDD did not differ significantly with healthy controls. The antidepressant dose or the Hamilton depression rating scale score did not significantly correlate with the oxytocin levels in MDD patients. CSF oxytocin levels in schizophrenic patients significantly negatively correlated with second generation antipsychotic dose ($r = -0.49$, $P = 0.010$) but not with first generation antipsychotic dose ($r = -0.13$, $P = 0.50$). A significant correlation was observed between oxytocin levels and negative subscale of PANSS ($r = -0.38$, $P = 0.050$). This correlation remained significant even after controlling for second generation antipsychotic dose ($r = -0.47$, $P = 0.016$).
Conclusions: We obtained no evidence of altered CSF oxytocin levels in patients with schizophrenia or those with MDD. However, lower oxytocin levels may be related to higher second generation antipsychotic dose and more severe negative symptoms in schizophrenia.

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1. Introduction

Oxytocin is produced in the supraoptic and paraventricular nuclei of hypothalamus and is secreted into the blood stream from the posterior pituitary. Its release is induced by a variety of stressful stimuli, including noxious stimuli, conditioned fear, and exposure to novel environments (Onaka, 2004). Accumulating evidence indicates that oxytocin plays an important role in social interactions (Lim and Young, 2006; Bartz et al., 2010). Deficits in social functioning observed in psychiatric disorders including schizophrenia (Couture et al., 2006; Sparks et al., 2010) and mood disorders (Inoue et al., 2004; Montag et al., 2010; Wolkenstein et al., 2011) imply the possible involvement of oxytocin in the pathophysiology of these disorders.

Many studies have investigated the possible link between oxytocin and psychiatric disorders. Some previous studies reported altered oxytocin function in patients with schizophrenia (Linkowski et al., 1984; Beckmann et al., 1985; Mai et al., 1993). Higher plasma oxytocin levels in schizophrenic patients were associated with lower symptom severity (Rubin et al., 2010). A clinical study showed that administration of this hormone ameliorated symptoms of schizophrenia (Feifel et al., 2010). In a preclinical study, systemically administered oxytocin reversed prepulse inhibition deficits induced by amphetamine and the phencyclidine analog in rats (Feifel and Reza, 1999). Oxytocin dysfunction has been implicated in the pathophysiology of depression as well. Two studies have shown that peripheral oxytocin levels and depressive symptoms were significantly correlated in patients with major depressive disorder (MDD) (Scantamburlo et al., 2007; Cyranowski et al., 2008). Moreover, oxytocin knock-out mice have shown dysregulated stress responses to psychological stimuli (Mantella et al., 2005) and enhanced anxiety behaviors (Mantella et al., 2003).

Oxytocin secreted from the pituitary gland generally does not re-enter the brain through the blood-brain barrier (Ermisch et al., 1985). Therefore, the behavioral effects of oxytocin are likely to be due to the release from centrally projecting oxytocin neurons. Since

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oxytocin in the nervous system can be transported to blood (Durham et al., 1991), peripheral oxytocin levels may reflect brain levels to some extent. However, central and peripheral oxytocin is regulated independently, and the half-life of oxytocin is less than 5 minutes in the blood (Ryden and Sjoholm, 1969) while that in the brain is 19.1 -minutes (Durham et al., 1991). Therefore, measurement in the CSF is necessary for the direct assessment of central oxytocin levels.

To our knowledge, two studies have previously examined the cerebrospinal fluid (CSF) levels of oxytocin in patients with schizophrenia. One reported elevated oxytocin levels in schizophrenia compared with controls (Beckmann et al., 1985), while the other did not obtain such a finding (Glovinsky et al., 1994). Only one study has examined the CSF levels of oxytocin in patients with depression, in which no difference was found compared with controls (Pitts et al., 1995). No study to date has examined the association of CSF oxytocin levels with symptom severity of these disorders. Since symptom severity forms a continuous spectrum ranging from mild to severe state, an association with the severity of the disease would suggest that oxytocin levels reflect the state of the disease.

In the present study, the oxytocin levels in the CSF of patients with schizophrenia and those with depression were measured and compared to that of healthy controls. Furthermore, we investigated the correlation between CSF oxytocin levels and symptom severity of these disorders. From the findings of previous studies examining peripheral oxytocin levels (Scantamburlo et al., 2007; Rubin et al., 2010), we hypothesized that CSF oxytocin levels would be lower in patient groups compared to healthy controls and that symptom severity would be negatively correlated with the oxytocin levels.

2. Materials and methods

2.1. Subjects

Participants were 27 patients with schizophrenia (mean age (standard deviation): 42.6 (8.5) years), 17 patients with major depressive disorder (MDD) (age: 39.5 (8.0) years), and 21 healthy controls (age: 38.3 (15.3) years). Demographic and clinical characteristics of the subjects are summarized in Table 1. All subjects were males to avoid gender effects and were biologically unrelated Japanese recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in free local information magazines and by our website announcement. None of the healthy controls were on psychotropic medication, while 70.6% of the patients with MDD were treated with antidepressant medication at the time of the study. Most of the schizophrenic patients were prescribed antipsychotic medication, and all of those prescribed antipsychotics were on the medication for more than 3 years. Consensus diagnosis by at least 2 psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria (American Psychiatric Association, 1994), on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers with no current or past history of psychiatric treatment and were screened using the Japanese version of the Mini International Neuropsychiatric Interview (Sheehan et al., 1998; Otsubo et al., 2005) by a research psychiatrist to eliminate the possibility of any axis I psychiatric disorders. Participants were excluded if they had prior medical histories of central nervous system diseases or severe head injury or if they met the criteria for substance abuse or dependence or mental retardation. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After describing the study, written informed consent was obtained from every subject.

2.2. Clinical measures

Schizophrenic symptoms and depressive symptoms were assessed immediately after the lumbar puncture by an experienced research psychiatrist using the Japanese version of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987; Yamada et al., 1991) and the Japanese version of the GRID Hamilton Depression Rating Scale, 17-item version (HAMD-17) (Hamilton, 1967), which have both been demonstrated to show good inter-rater reliability (Igarashi et al., 1998; Tabuse et al., 2007). Medication status at the time of lumbar puncture was recorded. Daily doses of antipsychotics in patients with schizophrenia and antidepressants in patients with MDD were

Table 1
Demographic and clinical characteristics.

	Controls ($N=21$)	Schizophrenia ($N=27$)	Depression ($N=17$)	Analysis
Age (years)	38.3 (15.3)	42.6 (8.5)	39.5 (8.0)	ANOVA: $F=0.97$, n.s.
BMI	23.9 (4.1)	26.0 (6.2)	23.9 (4.5)	ANOVA: $F=1.06$, n.s.
Duration of illness (years)		16.3 (9.8)	7.7 (7.3)	t-test: $t=2.8$, $P<0.01$
Treatment duration (years)		15.5 (9.1)	5.8 (6.9)	t-test: $t=3.4$, $P<0.01$
Medication status				
on antipsychotic medication				
first generation (%)	0	59.3	11.8	
second generation (%)	0	66.7	23.5	
first and/or second generation (%)	0	96.3	35.3	
on antidepressant medication (%)	0	25.9	70.6	
on benzodiazepine medication (%)	0	81.5	76.5	
on mood stabilizer medication (%)	0	14.8	5.9	
CP equivalent dose				
first generation (mg/day)		361.8 (445.0)		
second generation (mg/day)		402.4 (498.3)		
total (mg/day)		764.2 (591.6)		
IMI equivalent dose (mg/day)			167.2 (141.5)	
PANSS				
Positive symptoms score		12.5 (3.8)		
Negative symptom score		16.0 (5.8)		
General symptom score		6.8 (1.3)		
Total score		55.6 (12.6)		
HAMD-17 score			13.4 (9.6)	

Values are shown as mean (standard deviation).
BMI: body mass index; CP: chlorpromazine; IMI: imipramine.
PANSS: Positive and Negative Syndrome Scale; HAMD-17: 17 item Hamilton Rating Scale for Depression.
ANOVA: analysis of variance; n.s.: not significant.

converted to chlorpromazine and imipramine equivalent doses, respectively, using published guidelines (Inagaki et al., 1999).

2.3. Lumbar puncture and oxytocin assay

Lumbar puncture was performed with the subject in the left decubitus position. CSF was withdrawn from the L3–L4 or L4–L5 interspace. After the removal of 2 ml of CSF, a further 6 ml of CSF was collected and immediately transferred on ice to be centrifuged at 4 °C and aliquoted for storage at −80 °C until assay. CSF oxytocin levels were analyzed using a commercial ELISA kit (Enzo Life Sciences, INC., NY). Using the results from two separate runs of standard concentrations, the inter-assay coefficient of variation (CV) was less than 10%.

2.4. Statistical analysis

Statistical differences between groups were calculated using Student's *t*-test, Welch's *t*-test, or one-way analysis of variance (ANOVA). Correlations were assessed using Pearson's correlation coefficient. Since the CSF oxytocin levels were not normally distributed, log transformation was applied prior to statistical analyses to achieve normal distribution. Because previous studies suggest that some antipsychotic and antidepressant medications increase oxytocin secretion (Uvnas-Moberg et al., 1992, 1999), chlorpromazine and imipramine equivalent doses were examined as possible confounders. Statistical analyses were performed using the Statistical Package for the Social Sciences version 11.0 (SPSS Japan, Tokyo, Japan). All statistical tests were two-tailed, and $P<0.05$ indicated statistical significance.

3. Results

Fig. 1 shows the CSF oxytocin levels in each diagnostic group. A one-way ANOVA using the transformed oxytocin levels as the dependent variable indicated no significant difference between diagnostic groups ($F=1.08$, $P=0.35$). The transformed oxytocin levels showed no significant correlation with age or body weight. Figs. 2 and 3 show the relation of CSF oxytocin levels with symptom severity and psychotropic dose, respectively. The antidepressant dose or the HAMD-17 score did not significantly correlate with the transformed oxytocin levels in patients with MDD (antidepressant dose: $r=-0.15$, $P=0.57$; HAMD-17: $r=-0.19$, $P=0.46$). The transformed oxytocin levels were significantly negatively correlated with negative subscale of PANSS ($r=-0.38$, $P=0.050$). Correlations between transformed oxytocin levels and other subscales of PANSS were not statistically significant. The transformed oxytocin levels in schizophrenic patients were significantly negatively correlated with chlorpromazine equivalents of total antipsychotic dose ($r=-0.51$, $P=0.0064$) and second generation antipsychotic (SGA) dose ($r=-0.49$, $P=0.010$) but not with chlorpromazine equivalents of first generation antipsychotic (FGA) dose ($r=-0.13$, $P=0.50$). Those prescribed SGA had significantly lower CSF oxytocin levels compared to those not prescribed SGA (Welch's *t* test: $t=2.6$, $df=10.4$, $P=0.024$). Comparison between patients prescribed and not prescribed FGA did not yield significant difference (Student's t test: $t=1.1$, $df=25$, $P=0.27$). Although none of the subscales of PANSS were correlated with FGA, SGA, or total chlorpromazine equivalent dose in the present study (all $P>0.1$), a previous study (Sim et al., 2009) reported an association between antipsychotic dose and the severity of positive as well as negative symptoms of schizophrenia. Therefore, we considered antipsychotic dose as a confounding factor for the association between oxytocin levels and symptom severity. Thus, we also examined the correlation between the oxytocin levels and PANSS scores controlling for prescribed antipsychotic dose. Partial correlation between transformed oxytocin levels and negative subscale of PANSS, removing the linear effects of total antipsychotic dose, was statistically significant ($r=-0.39$, $P=0.047$). Removing the linear effects of SGA dose instead of total antipsychotic dose also resulted in significant correlation of transformed CSF oxytocin levels with negative subscale ($r=-0.47$, $P=0.016$) as well as with total PANSS score ($r=-0.47$, $P=0.016$). SGA dose-controlled partial correlations between transformed oxytocin levels and other subscales of PANSS were not statistically significant (positive subscale: $r=-0.24$, $P=0.23$; general subscale: $r=-0.33$, $P=0.099$).

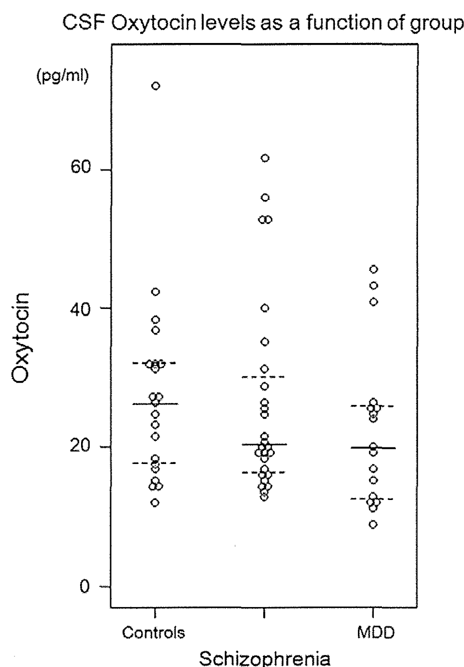

Fig. 1. Cerebrospinal fluid oxytocin levels as a function of group. The cerebrospinal fluid oxytocin levels in healthy controls and patients with schizophrenia and major depressive disorder are shown. Solid bars indicate median values and the dotted lines indicate interquartile range. No significant difference was observed between the diagnostic groups.

4. Discussion

Consistent with some previous studies (Glovinsky et al., 1994; Pitts et al., 1995), CSF oxytocin levels did not significantly differ between healthy controls and patients with schizophrenia and MDD. However, the present results showed that higher levels of CSF oxytocin may be associated with less severe symptoms of schizophrenia.

The observed negative correlation between antipsychotic dose and CSF oxytocin levels points to the possibility that antipsychotic medication lowers oxytocin levels. A recent study suggests that an inhibitory feedback loop may exist between prolactin-secreting lactrophs and oxytocinergic paraventricular neurons (Sirzen-Zelenskaya et al., 2011). Therefore, the disinhibition of prolactin secretion due to the D_2 receptor blockade by antipsychotics may have resulted in the suppression of oxytocin secretion. This, however, does not explain the stronger correlation of SGA dose compared to FGA dose. Kiss et al (2010) showed that SGAs have a more potent influence than haloperidol on the activity of oxytocin magnocellular neurons. This also seems contradictory to the present finding that SGA is negatively correlated with oxytocin levels. An alternative explanation for this negative correlation is that patients with low oxytocin levels may respond poorly to antipsychotic medication, and thus, higher dose was prescribed to such patients. Nevertheless, despite the relatively strong correlation with the antipsychotic dose, the cross-sectional design of the present study hinders any causal inferences. One previous study (Glovinsky et al., 1994) demonstrated that CSF oxytocin levels were unchanged by antipsychotic medication. Thus, further investigation is necessary to elucidate the effects of antipsychotic medication on oxytocin levels.

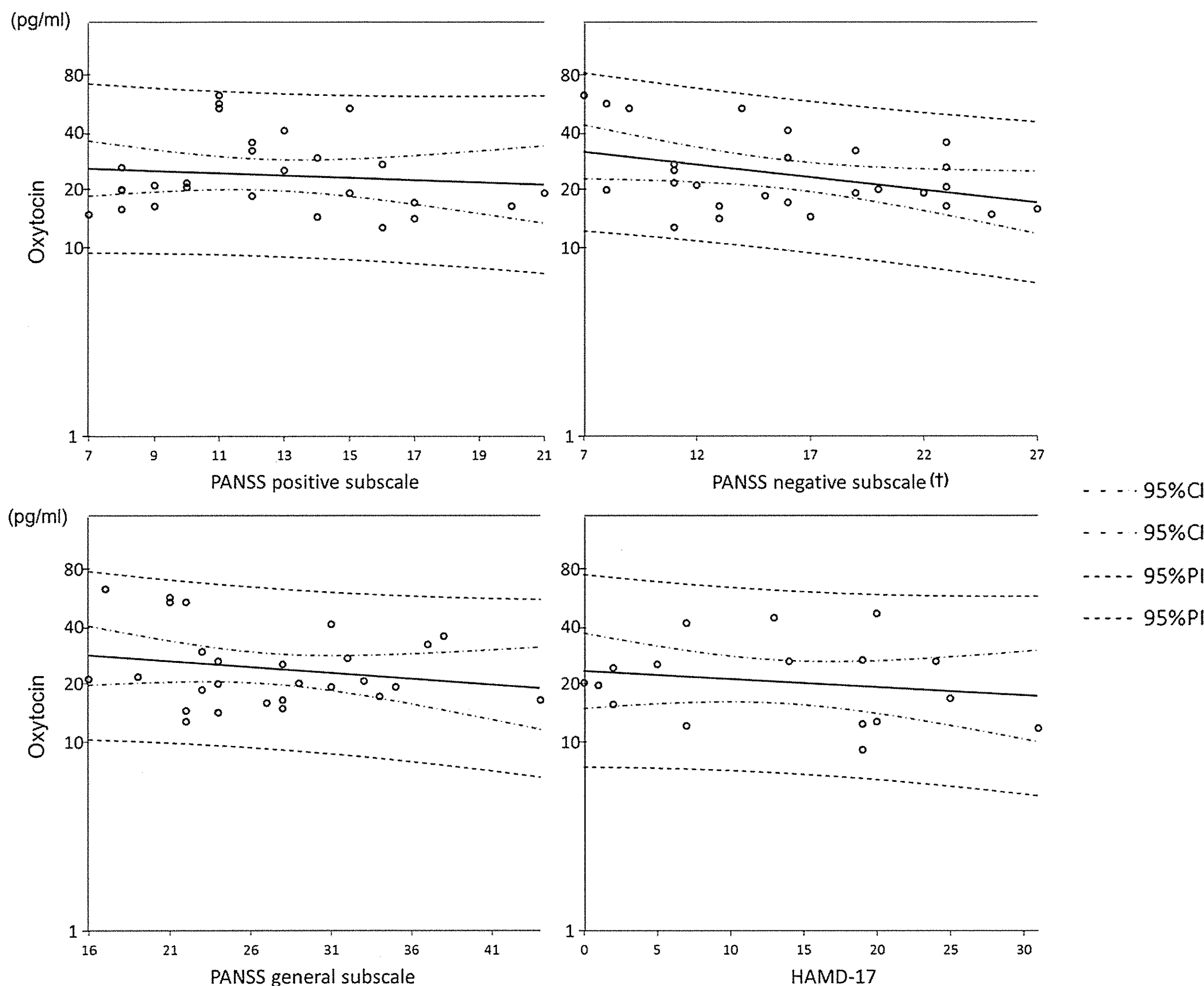

Fig. 2. Relationship between cerebrospinal fluid oxytocin levels and symptom severity. The association between cerebrospinal oxytocin levels and symptom severity is shown. Oxytocin levels are shown in logarithmic scale. Solid lines indicate fitted regression lines, unevenly dashed lines indicate 95% confidence intervals, and evenly dashed lines indicate 95% prediction intervals. (†): Correlation at significance level of $P<0.05$. PANSS: Positive and Negative Syndrome Scale, HAMD-17: Hamilton Depression Rating Scale, 17-item version, 95%CI: 95% confidence interval, 95%PI: 95% prediction interval.

The present results showed that the negative symptoms of schizophrenia were negatively correlated with CSF oxytocin levels. The correlation coefficient between CSF oxytocin levels and total PANSS score was also significant, controlling for SGA dose. Rubin et al. (2010) reported that higher peripheral oxytocin levels were associated with more prosocial behaviors in female patients with schizophrenia. Furthermore, previous studies have demonstrated improvement of social behaviors with administration of intranasal oxytocin (Macdonald and Macdonald, 2010; Pedersen et al., 2011). Since strong relationships between negative symptoms and social difficulties have been demonstrated in schizophrenia (Weinberg et al., 2009), the present finding associating higher CSF oxytocin levels with lower negative subscale is in accord with what has previously been described for peripheral oxytocin. Whether the peripheral oxytocin levels reflect the CSF oxytocin levels, or whether a different mechanisms of action in the brain and the peripheral result in a similar effect, remains to be explored.

Previous studies examining CSF oxytocin levels in patients with schizophrenia (Beckmann et al., 1985; Glovinsky et al., 1994) and depression (Pitts et al., 1995) showed mean oxytocin levels of less than 10 pg/ml, which is lower than that in the present study (>20 pg/ml). Such outcome may have resulted from some of the methodological differences between previous studies and the present one. Previous three studies measured oxytocin levels using radioimmunoassay (RIA), while the present study used a commercially available ELISA kit. A recent study that used the same ELISA kit to measure CSF oxytocin levels (Heim et al., 2009) also demonstrated higher levels of oxytocin (mean oxytocin levels of 17 pg/ml in women without a history of emotional abuse) compared to the previous studies using RIA. Thus, the different measurement techniques may have influenced the values.

A number of other methodological differences exist between the present study and previous ones examining CSF oxytocin levels (Beckmann et al., 1985; Glovinsky et al., 1994; Pitts et al., 1995). One of the major differences was that the present study did not require fasting prior to lumbar puncture, while Beckmann et al (Beckmann et al., 1985) collected CSF in patients with schizophrenia after 12 hours fasting. Although a previous study (Challinor et al., 1994) reported that peripheral oxytocin levels were not affected by 20 hours of fasting, the influence of fasting on CSF levels is unknown. Furthermore, Beckmann et al used Research Diagnostic Criteria to select a patient group consisting entirely of paranoid schizophrenia. Such difference in composition of participants may have affected the outcome of the study by Beckmann et al (1985), which showed significantly higher CSF oxytocin levels in schizophrenic patients compared to healthy controls. The findings by Glovinsky et al (1994) and Pitts et al (1995) were consistent with the present study in that no significant difference in CSF oxytocin levels was found between patients and controls. However,

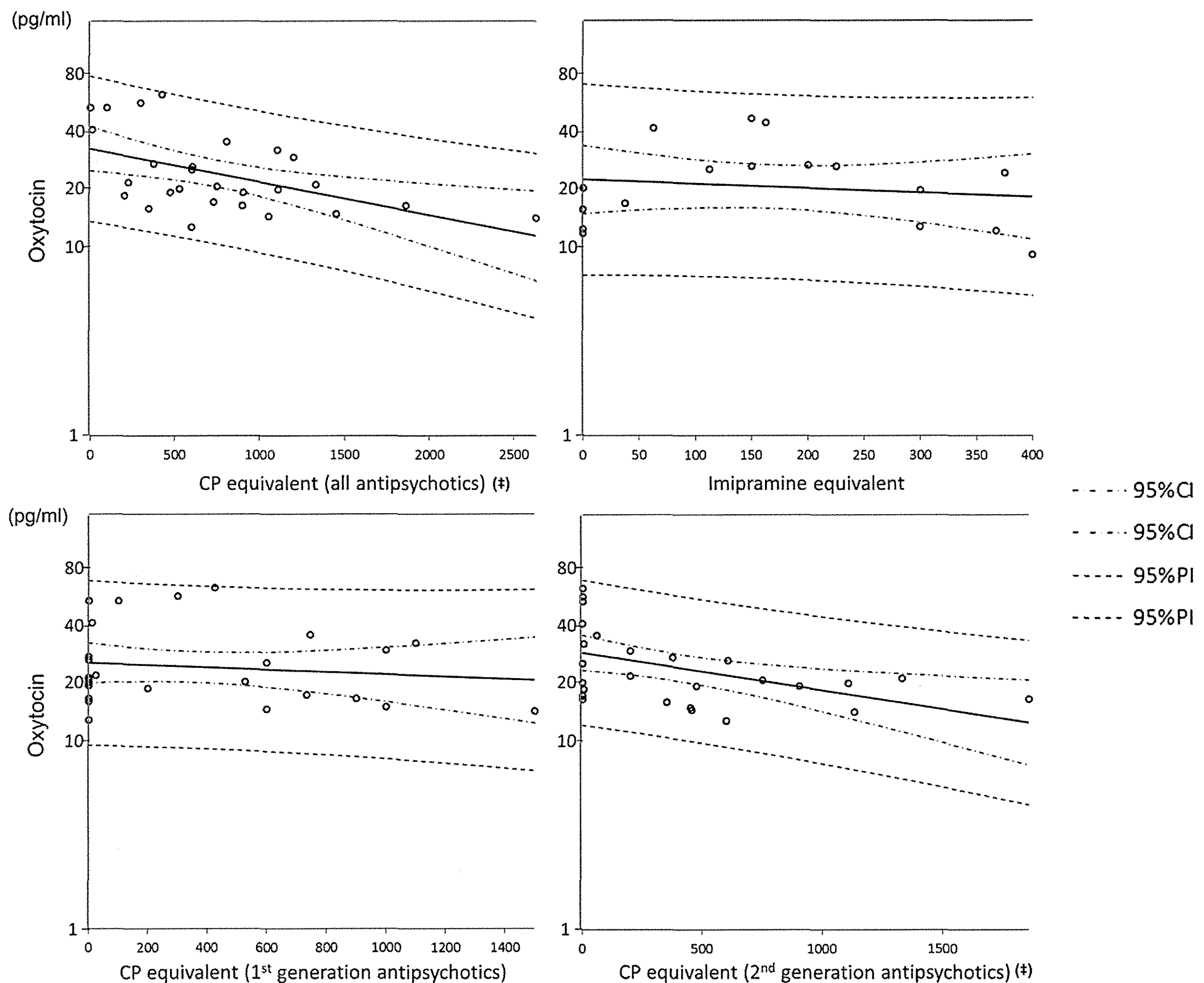

Fig. 3. Relationship between cerebrospinal fluid oxytocin levels and dose of psychotropics. The association between cerebrospinal oxytocin levels and dose of psychotropics is shown. Oxytocin levels are shown in logarithmic scale. Solid lines indicate fitted regression lines, unevenly dashed lines indicate 95% confidence intervals, and evenly dashed lines indicate 95% prediction intervals. (‡): Correlation at significance level of $P<0.01$. CP equivalent: chlorpromazine equivalent, 95%CI: 95% confidence interval, 95%PI: 95% prediction interval.

participants in these studies also differed from that of the present study in that both genders were included. Furthermore, MDD patients in the study by Pitts et al (1995) all scored 18 or above on the HAMD-17, while the MDD patients in the present study included those in a remitted state. These differences in composition of study samples should be carefully considered when comparing findings across studies.

Some limitations must be considered when interpreting the results of this study. First, the effects of medication could not be fully controlled due to the variability in types and doses. Future studies should examine oxytocin levels in untreated patients to elucidate the role of oxytocin in the pathophysiology of schizophrenia and depression. Treatment duration may also affect oxytocin levels. However, since all of the schizophrenic patients that were prescribed antipsychotics were on chronic treatment with the medication, treatment duration is unlikely to have confounded the main findings of the present study. Secondly, as mentioned above, the cross-sectional design did not allow for any definitive conclusions regarding the causal relationship between the CSF oxytocin levels, psychotropic medication, and symptom severity. Thirdly, only male participants were included in the present study. Previous studies suggest that effects of peripheral and intranasal oxytocin may differ between men and women (Domes et al., 2010; Rubin et al., 2010, 2011). Therefore, the present findings cannot be generalized to women. Finally, the risk of type II error was high due to the small sample size. The sample size in the present study was comparable to those of the previous studies that examined CSF oxytocin levels in patients with schizophrenia and depression (Beckmann et al., 1985; Glovinsky et al., 1994; Pitts et al., 1995). However, the power to detect a moderate difference (effect size of 0.50) in CSF oxytocin levels between patients and controls was relatively low (schizophrenia: 39%; MDD: 32%; calculated by G*Power 3.1.3 (Faul et al., 2007)). A larger sample may be necessary to detect small to moderate change in CSF oxytocin levels in psychiatric disorders.

In conclusion, we obtained no evidence of altered CSF oxytocin levels in patients with schizophrenia or those with MDD. However, lower CSF oxytocin levels may be related to higher SGA dose and more severe negative symptoms in schizophrenia, which is in line with the possibility that central oxytocin may ameliorate the severity of some symptoms of schizophrenia by improving social functioning.

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no role in the study design; the collection, analysis and interpretation of data, in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Daimei Sasayama and Kotaro Hattori designed the study. Daimei Sasayama, Kotaro Hattori, and Toshiya Teraishi performed the lumbar punctures. Daimei Sasayama, Kotaro Hattori, Toshiya Teraishi, Hiroaki Hori, Miho Ota, Sumiko Yoshida, Kunimasa Arima, and Hiroshi Kunugi screened and diagnosed the study participants. Daimei Sasayama wrote the draft of the manuscript. Hiroshi Kunugi supervised the writing of the paper. Teruhiko Higuchi and Naoji Amano gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest statement

The authors declare no conflicts of interest.

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References

American Psychiatric Association, 1994. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th edition. American Psychiatric Press, Washington D.C.

Bartz, J.A., Zaki, J., Ochsner, K.N., Bolger, N., Kolevzon, A., Ludwig, N., Lydon, J.E., 2010. Effects of oxytocin on recollections of maternal care and closeness. Proc. Natl. Acad. Sci. U. S. A. 107 (50), 21371–21375.

Beckmann, H., Lang, R.E., Gattaz, W.F., 1985. Vasopressin–oxytocin in cerebrospinal fluid of schizophrenic patients and normal controls. Psychoneuroendocrinology 10 (2), 187–191.

Challinor, S.M., Winters, S.J., Amico, J.A., 1994. Pattern of oxytocin concentrations in the peripheral blood of healthy women and men: effect of the menstrual cycle and short-term fasting. Endocr. Res. 20 (2), 117–125.

Couture, S.M., Penn, D.L., Roberts, D.L., 2006. The functional significance of social cognition in schizophrenia: a review. Schizophr. Bull. 32 (Suppl. 1), S44–S63.

Cyranowski, J.M., Hofkens, T.L., Frank, E., Seltman, H., Cai, H.M., Amico, J.A., 2008. Evidence of dysregulated peripheral oxytocin release among depressed women. Psychosom. Med. 70 (9), 967–975.

Domes, G., Lischke, A., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., Herpertz, S.C., 2010. Effects of intranasal oxytocin on emotional face processing in women. Psychoneuroendocrinology 35 (1), 83–93.

Durham, D.A., Banks, W.A., Kastin, A.J., 1991. Carrier-mediated transport of labeled oxytocin from brain to blood. Neuroendocrinology 53 (5), 447–452.

Ermisch, A., Barth, T., Ruhle, H.J., Skopkova, J., Hrbas, P., Landgraf, R., 1985. On the blood–brain barrier to peptides: accumulation of labelled vasopressin, DesGlyNH2-vasopressin and oxytocin by brain regions. Endocrinol. Exp. 19 (1), 29–37.

Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39 (2), 175–191.

Feifel, D., Reza, T., 1999. Oxytocin modulates psychotomimetic-induced deficits in sensorimotor gating. Psychopharmacology (Berl.) 141 (1), 93–98.

Feifel, D., Macdonald, K., Nguyen, A., Cobb, P., Warlan, H., Galangue, B., Minassian, A., Becker, O., Cooper, J., Perry, W., Lefebvre, M., Gonzales, J., Hadley, A., 2010. Adjunctive intranasal oxytocin reduces symptoms in schizophrenia patients. Biol. Psychiatry 68 (7), 678–680.

Glovinsky, D., Kalogeras, K.T., Kirch, D.G., Suddath, R., Wyatt, R.J., 1994. Cerebrospinal fluid oxytocin concentration in schizophrenic patients does not differ from control subjects and is not changed by neuroleptic medication. Schizophr. Res. 11 (3), 273–276.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. Br. J. Soc. Clin. Psychol. 6 (4), 278–296.

Heim, C., Young, L.J., Newport, D.J., Mletzko, T., Miller, A.H., Nemeroff, C.B., 2009. Lower CSF oxytocin concentrations in women with a history of childhood abuse. Mol. Psychiatry 14 (10), 954–958.

Igarashi, Y., Hayashi, N., Yamashina, M., Otsuka, N., Kuroki, N., Anzai, N., Kazamatsuri, H., 1998. Interrater reliability of the Japanese version of the Positive and Negative Syndrome Scale and the appraisal of its training effect. Psychiatry Clin. Neurosci. 52 (5), 467–470.

Inagaki, A., Inada, T., Fujii, Y., Yagi, G., 1999. Equivalent Dose of Psychotropics. Seiwa Shoten, Tokyo.

Inoue, Y., Tonooka, Y., Yamada, K., Kanba, S., 2004. Deficiency of theory of mind in patients with remitted mood disorder. J. Affect. Disord. 82 (3), 403–409.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261–276.

Kiss, A., Bundzikova, J., Pirnik, Z., Mikkelsen, J.D., 2010. Different antipsychotics elicit different effects on magnocellular oxytocinergic and vasopressinergic neurons as revealed by Fos immunohistochemistry. J. Neurosci. Res. 88 (3), 677–685.

Lim, M.M., Young, L.J., 2006. Neuropeptidergic regulation of affiliative behavior and social bonding in animals. Horm. Behav. 50 (4), 506–517.

Linkowski, P., Geenen, V., Kerkhofs, M., Mendlewicz, J., Legros, J.J., 1984. Cerebrospinal fluid neurophysins in affective illness and in schizophrenia. Eur. Arch. Psychiatry Neurol. Sci. 234 (3), 162–165.

Macdonald, K., Macdonald, T.M., 2010. The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. Harv. Rev. Psychiatry 18 (1), 1–21.

Mai, J.K., Berger, K., Sofroniew, M.V., 1993. Morphometric evaluation of neurophysin-immunoreactivity in the human brain: pronounced inter-individual variability and evidence for altered staining patterns in schizophrenia. J. Hirnforsch. 34 (2), 133–154.

Mantella, R.C., Vollmer, R.R., Li, X., Amico, J.A., 2003. Female oxytocin-deficient mice display enhanced anxiety-related behavior. Endocrinology 144 (6), 2291–2296.

Mantella, R.C., Vollmer, R.R., Amico, J.A., 2005. Corticosterone release is heightened in food or water deprived oxytocin deficient male mice. Brain Res. 1058 (1–2), 56–61.

Montag, C., Ehrlich, A., Neuhaus, K., Dziobek, I., Heekeren, H.R., Heinz, A., Gallinat, J., 2010. Theory of mind impairments in euthymic bipolar patients. J. Affect. Disord. 123 (1–3), 264–269.

Onaka, T., 2004. Neural pathways controlling central and peripheral oxytocin release during stress. J. Neuroendocrinol. 16 (4), 308–312.

Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., Kamijima, K., 2005. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry Clin. Neurosci. 59 (5), 517–526.

Pedersen, C.A., Gibson, C.M., Rau, S.W., Salimi, K., Smedley, K.L., Casey, R.L., Leserman, J., Jarskog, L.F., Penn, D.L., 2011. Intranasal oxytocin reduces psychotic symptoms and improves Theory of Mind and social perception in schizophrenia. Schizophr. Res. 132 (1), 50–53.

Pitts, A.F., Samuelson, S.D., Meller, W.H., Bissette, G., Nemeroff, C.B., Kathol, R.G., 1995. Cerebrospinal fluid corticotropin-releasing hormone, vasopressin, and oxytocin concentrations in treated patients with major depression and controls. Biol. Psychiatry 38 (5), 330–335.

Rubin, L.H., Carter, C.S., Drogos, L., Pournajafi-Nazarloo, H., Sweeney, J.A., Maki, P.M., 2010. Peripheral oxytocin is associated with reduced symptom severity in schizophrenia. Schizophr. Res. 124 (1–3), 13–21.

Rubin, L.H., Carter, C.S., Drogos, L., Jamadar, R., Pournajafi-Nazarloo, H., Sweeney, J.A., Maki, P.M., 2011. Sex-specific associations between peripheral oxytocin and emotion perception in schizophrenia. Schizophr. Res. 130 (1–3), 266–270.

Ryden, G., Sjoholm, I., 1969. Half-life of oxytocin in blood of pregnant and non-pregnant women. Acta Endocrinol. (Copenh.) 61 (3), 425–431.

Scantamburlo, G., Hansenne, M., Fuchs, S., Pitchot, W., Marechal, P., Pequeux, C., Ansseau, M., Legros, J.J., 2007. Plasma oxytocin levels and anxiety in patients with major depression. Psychoneuroendocrinology 32 (4), 407–410.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl. 20), 22–33 quiz 34-57.

Sim, K., Su, H.C., Fujii, S., Yang, S.Y., Chong, M.Y., Ungvari, G., Si, T., He, Y.L., Chung, E.K., Chan, Y.H., Shinfuku, N., Kua, E.H., Tan, C.H., Sartorius, N., 2009. High-dose antipsychotic use in schizophrenia: a comparison between the 2001 and 2004 Research on East Asia Psychotropic Prescription (REAP) studies. Br. J. Clin. Pharmacol. 67 (1), 110–117.

Sirzen-Zelenskaya, A., Gonzalez-Iglesias, A.E., Boutet de Monvel, J., Bertram, R., Freeman, M.E., Gerber, U., Egli, M., 2011. Prolactin induces a hyperpolarising current in rat paraventricular oxytocinergic neurones. J. Neuroendocrinol. 23 (10), 883–893.

Sparks, A., McDonald, S., Lino, B., O'Donnell, M., Green, M.J., 2010. Social cognition, empathy and functional outcome in schizophrenia. Schizophr. Res. 122 (1–3), 172–178.

Tabuse, H., Kalali, A., Azuma, H., Ozaki, N., Iwata, N., Naitoh, H., Higuchi, T., Kanba, S., Shioe, K., Akechi, T., Furukawa, T.A., 2007. The new GRID Hamilton Rating Scale for Depression demonstrates excellent inter-rater reliability for inexperienced and experienced raters before and after training. Psychiatry Res. 153 (1), 61–67.

Uvnas-Moberg, K., Alster, P., Svensson, T.H., 1992. Amperozide and clozapine but not haloperidol or raclopride increase the secretion of oxytocin in rats. Psychopharmacology (Berl.) 109 (4), 473–476.

Uvnas-Moberg, K., Bjokstrand, E., Hillegaart, V., Ahlenius, S., 1999. Oxytocin as a possible mediator of SSRI-induced antidepressant effects. Psychopharmacology (Berl.) 142 (1), 95–101.

Weinberg, D., Shahar, G., Davidson, L., McGlashan, T.H., Fennig, S., 2009. Longitudinal associations between negative symptoms and social functioning in schizophrenia: the moderating role of employment status and setting. Psychiatry 72 (4), 370–381.

Wolkenstein, L., Schonenberg, M., Schirm, E., Hautzinger, M., 2011. I can see what you feel, but I can't deal with it: impaired theory of mind in depression. J. Affect. Disord. 132 (1–2), 104–111.

Yamada, H., Masui, K., Kikuimoto, K., 1991. The Japanese version of The Positive and Negative Syndrome Scale (PANSS) Rating Manual. Seiwa, Tokyo.

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Proteomic Analysis of Lymphoblastoid Cells Derived from Monozygotic Twins Discordant for Bipolar Disorder: A Preliminary Study

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Abstract

Bipolar disorder is a severe mental illness characterized by recurrent manic and depressive episodes. In bipolar disorder, family and twin studies suggest contributions from genetic and environmental factors; however, the detailed molecular pathogenesis is yet unknown. Thus, identification of biomarkers may contribute to the clinical diagnosis of bipolar disorder. Monozygotic twins discordant for bipolar disorder are relatively rare but have been reported. Here we performed a comparative proteomic analysis of whole cell lysate derived from lymphoblastoid cells of monozygotic twins discordant for bipolar disorder by using two-dimensional differential in-gel electrophoresis (2D-DIGE). We found approximately 200 protein spots to be significantly differentially expressed between the patient and the co-twin (*t* test, $p<0.05$). Some of the proteins were subsequently identified by liquid chromatography tandem mass spectrometry and included proteins involved in cell death and glycolysis. To examine whether these proteins could serve as biomarkers of bipolar disorder, we performed Western blot analysis using case–control samples. Expression of phosphoglycerate mutase 1 (PGAM1), which is involved in glycolysis, was significantly up-regulated in patients with bipolar disorder (*t* test, $p<0.05$). Although PGAM1 cannot be regarded as a qualified biomarker of bipolar disorder from this preliminary finding, it could be one of the candidates for further study to identify biomarkers of bipolar disorder.

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Introduction

Bipolar disorder is a severe mental illness characterized by recurrent manic and depressive episodes. It affects approximately 1% of the general population. In bipolar disorder, contributions of genetic and environmental factors have been indicated by family and twin studies [1]. Patients with this disease have a high relapse rate, and lifelong treatment with mood stabilizers such as lithium is often needed [2]. Past studies of bipolar disorder have focused on monoamines and intracellular signal transduction pathways as related to the effects of psychotropic drugs [3,4], but recent studies focus more on neuroplasticity or resilience based on reports of neuroprotective effects of mood stabilizers [5]. Recent genome-wide association studies identified new candidate genes, such as *CACNA1C* [6,7], but the effects are relatively small.

Bipolar disorder is often misdiagnosed as depression or schizophrenia, and delayed diagnosis and treatment worsen the course of illness [8]. Thus, early diagnosis is important to prevent deterioration; however, no biomarkers for bipolar disorder are available yet. Identification of biomarkers would be indispensable for early diagnosis.

Much research on biomarkers in psychiatric diseases has been published [9,10]. Though several candidate biomarkers were identified from postmortem brains, these studies are undermined by many confounding factors, including cause of death, postmortem interval, and brain pH [11]. Postmortem interval affects degradation of mRNAs and protein levels [12,13,14,15] and significantly influences phosphorylation of signaling proteins [16]. In particular, the influence of medication cannot be ignored because a number of proteins are affected by mood stabilizers, antipsychotics, or antidepressant medication [17,18,19]. Additionally, large interindividual variations hamper the identification of biomarkers.

Biomarker research in other tissues such as serum, plasma, cerebrospinal fluids, saliva, and urine have also been performed [20,21] but has not led to a diagnostic test.

The concordance rate of bipolar disorder between monozygotic twins is approximately 70% [22,23]. By comparing monozygotic twins discordant for bipolar disorder, biochemical differences associated with bipolar disorder might be detected without interference from interindividual genetic variation [24].

Although several genome, transcriptome, and epigenome analyses in monozygotic twins discordant for bipolar disorder and other diseases have been reported [24–29], proteomic analysis has not been applied to identifying the difference between

monozygotic twins discordant for bipolar disorder, possibly due to technical difficulties. Because transcript levels do not completely correlate with protein expression levels [30,31] and aberrant post-translational modifications can cause disease, proteomic analysis is needed to supplement transcriptome and epigenome analyses.

In the present study, we performed proteomic analysis of lymphoblastoid cells derived from monozygotic twins discordant for bipolar disorder.

Materials and Methods

Subjects

For 2D-DIGE, we used lymphoblastoid cells derived from a pair of 42-year-old male monozygotic twins discordant for bipolar disorder. We initially examined their monozygosity by genotyping microsatellite repeat markers [25] and later confirmed it by single nucleotide polymorphism array [29].

For a case–control study of differentially expressed proteins using Western blot analyses, we used lymphoblastoid cells derived from eight unrelated patients with bipolar I disorder (BPI) (four men and four women, 35.6±9.0 years old [mean ± SD], Japanese) and eight unrelated control subjects (six men and two women, 36.9±10.0 years old [mean ± SD], Japanese). Table 1 gives detailed information for each subject used in Western blot analysis. Patients and controls with a history of alcohol or illicit drug abuse were excluded from the study. The patients were treated with various medications. Diagnoses were made by the consensus of two senior psychiatrists using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. Controls were selected from among students, nurses, office workers, and doctors in participating institutes, their friends, and other volunteers. A senior psychiatrist interviewed control subjects and found that they did not have major mental disorders. Written informed consent was obtained from all subjects. The ethics committees of RIKEN approved the study.

Cell culture and extraction of proteins

Lymphocytes were separated from the peripheral blood and transformed by Epstein-Barr virus using previously described standard techniques [32]. These cells were cultured and kept frozen until experiments. Lymphoblastoid cells were cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (Sigma-Aldrich), 50 U/mL penicillin, 50 µg/mL streptomycin (GIBCO, Invitrogen/Life Technologies Corporation, Grand Island, NY), and 60 µg/mL tylosin solution (Sigma-Aldrich). Cells were cultured at approximately 1×10^8 cells. Total proteins were extracted from the lymphoblastoid cells using the Q-proteome mammalian protein preparation kit (Qiagen, QIAGEN, Hilden, Germany). After the supernatant was precipitated with acetone, the pellet was dissolved using the lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 10 mM Tris-HCl [pH 8.5]) (GE Healthcare Bio-Sciences, San Francisco, CA, USA).

Two-dimensional difference gel electrophoresis (2D-DIGE) and imaging analyses

Protein concentration was determined by the Bradford method using a protein assay kit (Bio-Rad, Hercules, CA, USA) and the Pierce 660 nm protein assay (Pierce, Rockford, IL, USA). Proteins were set to a final concentration of 5 mg/mL with the lysis buffer and labeled separately with 400 pmol of CyDye (Cy3 or Cy5) (GE Healthcare Bio-Sciences), vortexed, and incubated on ice in the dark for 30 min. A mixed sample composed of equal amount of proteins from the patient and the co-twin was labeled with Cy2 and used as an internal standard. After 30 min, the labeling reaction was stopped with 10 mM lysine. To avoid the possible effect of labeling efficiency, the dyes were swapped for each experiment using three gels. Labeled proteins were subjected to SDS-PAGE analysis, and the gels were scanned with the Typhoon 9400 scanner (GE Healthcare Bio-Sciences) at the wavelengths

Table 1. Characteristics of the subjects.

ID	Age	Sex	Diagnosis	Age at onset	Family history of bipolar disorder within first degree relatives	Medication
B1	40	F	Bipolar I disorder with psychotic features	24	Yes	Li, VPA, CBZ, HP, CP
B2	23	F	Bipolar I disorder	23	No	Li, VPA, PAR
B3	46	F	Bipolar I disorder	27	Yes	VPA, THY
B4	26	M	Bipolar I disorder, rapid cycling	24	Yes	Li
B5	38	M	Bipolar I disorder	35	No	VPA, OLA
B6	40	M	Bipolar I disorder	25	No (three had major depression)	Li, VPA
B7	27	F	Bipolar I disorder with psychotic features	24	No	Li, VPA
B8	45	M	Bipolar I disorder	20	No	Li
C1	36	M	-		No	-
C2	29	M	-		No	-
C3	34	M	-		No	-
C4	29	M	-		No	-
C5	27	F	-		No	-
C6	52	M	-		No	-
C7	52	M	-		No	-
C8	33	F	-		No	-

Li: lithium carbonate, VPA, sodium valproate, CBZ, carbamazepine, HP, haloperidol, CP, chlorpromazine, PAR, paroxetine, THY, levothyroxine, OLA, olanzapine.
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Table 2. Proteins differentially expressed in monozygotic twin discordant for BP identifed by LC-MS/MS.

			Exp.1		Exp.2		Exp.3		Exp.4	
			Fold change		Fold change		Fold change		Fold change	
Gene names	**Acc. No.**	**Protein names**	**(BP/CT)**	***p* – value**	**(BP/CT)**	***p* – value**	**(BP/CT)**	***p* – value**	**(BP/CT)**	***p* – value**
PSME1	Q06323	Proteasome activator complex subunit 1	−1.42	0.00013	−1.44	0.00018	−1.3	0.0079	−1.91	0.0056
RPLP0	P05388	60S acidic ribosomal protein P0	−1.34	0.00031	−1.39	0.03	−1.45	0.0053	−1.31	0.0059
TPI1	P00939	Triosephosphate isomerase	1.95	0.034	3.17	7.10E-05	1.68	0.015	1.3	0.015
ALDOC	P09972	Fructose-bisphosphate aldolase C	−1.47	0.0024	−1.35	0.0013	−1.26	0.0059		
ANXA4	P09525	Annexin A4	−1.96	0.00022	−1.62	0.00011	−1.35	0.00029		
PGAM1	P18669	Phosphoglycerate mutase 1	1.9	0.0023	1.59	0.0001			2.12	0.0035
WARS	P23381	Tryptophanyl-tRNA synthetase, cytoplasmic	1.6	0.00037	1.55	0.00023			1.28	0.00062
ACADS	P16219	Short-chain specific acyl-CoA dehydrogenase, mitochondrial					−1.56	0.00014	−1.37	0.0006
ALDH2	P05091	Aldehyde dehydrogenase, mitochondrial					−1.26	0.018	−1.29	0.01
ALDOA	P04075	Fructose-bisphosphate aldolase A			1.48	0.0023			1.29	0.0055
ANXA5	P08758	Annexin A5					−1.25	0.0046	−1.29	0.007
APOA1BP	Q8NCW5	Apolipoprotein A-I-binding protein	2.56	1.90E-06	2.16	0.00023				
ATP5A1	P25705	ATP synthase subunit alpha, mitochondrial	−1.61	0.00048			−1.33	6.70E-06		
C19orf10	Q969H8	UPF0556 protein C19orf10	1.66	0.001	2.16	0.0012				
CACYBP	Q9HB71	Calcyclin-binding protein			−1.99	0.00075	−11.74	0.00023		
CAPZB	P47756	F-actin-capping protein subunit beta	−1.56	0.00021	−1.36	7.10E-06				
CASP3	P42574	Caspase-3	1.49	0.00011	1.44	0.0054				
CMPK1	P30085	UMP-CMP kinase	2.9	1.40E-05	3.22	0.00029				
CORO1A	P31146	Coronin-1A	1.31	0.0013	1.36	0.0029				
DNAJB11	Q9UBS4	DnaJ homolog subfamily B member 11	1.37	0.00032	1.41	0.0052				
ECH1	Q13011	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	1.91	0.0014	1.74	0.00016				
ENO1	P06733	Alpha-enolase	−1.62	0.049	−1.58	0.012				
ETFB	P38117	Electron transfer flavoprotein subunit beta	1.27	0.019	1.36	0.00099				
GALE	Q14376	UDP-glucose 4-epimerase	−1.35	0.0051			−1.33	0.044		
GAPDH	P04406	Glyceraldehyde-3-phosphate dehydrogenase	−1.38	0.0038			−1.33	0.044		
HIST2H4B	P62805	Histone H4	2.07	0.0038	1.95	0.0014				
HNRNPM	P52272	Heterogeneous nuclear ribonucleoprotein M					1.46	0.0033	1.4	0.0023
HSPA5	P11021	78 kDa glucose-regulated protein	3.18	0.00012	3.02	5.50E-06				
HSPB1	P04792	Heat shock protein beta-1	1.58	0.0001	1.46	0.0071				
LDHA	P00338	L-lactate dehydrogenase A chain			1.26	0.035			5.6	0.0042
LGALS3	P17931	Galectin-3	−1.78	0.00033	−1.99	0.00075				
NANS	Q9NR45	Sialic acid synthase	1.46	0.0012	1.62	1.60E-05				
NDUFS3	O75489	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	1.58	0.0001	1.46	0.0071				
NPM1	P06748	Nucleophosmin	−1.53	0.00074	−1.42	0.0042				
OTUB1	Q96FW1	Ubiquitin thioesterase OTUB1	1.52	6.00E-05	1.47	0.0043				
P4HB	P07237	Protein disulfide-isomerase	1.47	0.00017	1.69	0.0029				
PACAP	Q8WU39	Plasma cell-induced resident endoplasmic reticulum protein	2	0.011	2.01	0.0077				
PCBP1	Q15365	Poly(rC)-binding protein 1	−1.47	0.0024	−1.35	0.0013				

Table 2. Cont.

			Exp.1		Exp.2		Exp.3		Exp.4	
			Fold change		**Fold change**		**Fold change**		**Fold change**	
Gene names	**Acc. No.**	**Protein names**	**(BP/CT)**	***p* – value**	**(BP/CT)**	***p* – value**	**(BP/CT)**	***p* – value**	**(BP/CT)**	***p* – value**
PDIA3	P30101	Protein disulfide-isomerase A3	1.98	8.60E-06	2.09	0.0017				
PDIA6	Q15084	Protein disulfide-isomerase A6	1.58	0.00042	1.75	1.20E-05				
PHGDH	O43175	D-3-phosphoglycerate dehydrogenase	2.05	0.003	3.13	0.0001				
PITHD1	Q9GZP4	PITH domain-containing protein 1	2.15	0.012	2.13	0.011				
PKM2	P14618	Pyruvate kinase isozymes M1/M2			1.36	0.0029			1.36	0.045
PNPO	Q9NVS9	Pyridoxine-5′-phosphate oxidase	−1.74	0.0085	−1.41	0.001				
POLR2E	P19388	DNA-directed RNA polymerases I, II, and III subunit RPABC1	1.65	0.00025	2.12	0.00013				
PRDX2	P32119	Peroxiredoxin-2	2.9	1.40E-05	3.22	0.00029				
PSMB1	P20618	Proteasome subunit beta type-1	−1.77	0.0093			−1.29	0.00032		
SARS	P49591	Seryl-tRNA synthetase, cytoplasmic	1.31	0.0013	1.36	0.0029				
SERPINB1	P30740	Leukocyte elastase inhibitor	1.37	0.00032	1.41	0.0052				
SSR4	P51571	Translocon-associated protein subunit delta	1.67	0.0059	1.77	0.00018				
STMN1	P16949	Stathmin	−2.02	6.90E-05	−1.53	3.70E-05				
UCHL1	P09936	Ubiquitin carboxyl-terminal hydrolase isozyme L1	2.5	1.90E-05	2.16	0.00023				
VDAC1	P21796	Voltage-dependent anion-selective channel protein 1	1.43	0.0013	2.29	0.00023				

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corresponding to each CyDye, namely 480 nm (Cy2), 532 nm (Cy3), and 633 nm (Cy5). A 50 µg portion of each Cy3-, Cy5-, and Cy2-labeled sample was combined. Nonlinear IPG strips (pH 3–10, 18 cm long; GE Healthcare Bio-Sciences) were rehydrated for 12 h at 50 mA per strip with the sample solution on an IPGphor isoelectric focusing unit (GE Healthcare Bio-Sciences). Three mixed samples were separated by isoelectric focusing on an IPGphor isoelectric focusing unit at 0.5 V·h, 0.8 V·h, 13.5 kV·h, and 21 kV·h at 20°C and a maximum current setting of 50 µA per strip. The second dimension was run on 12.5% acrylamide gels in a SE600 (GE Healthcare Bio-Sciences) at 45 mA per gel. To avoid artifacts, three gels of same condition were simultaneously run for each experiment. Gels were scanned directly between low-fluorescence glass plates with the Typhoon 9400 (GE Healthcare Bio-Sciences) scanner at the three wavelengths specific for the CyDyes. The resolution was approximately 100 µm. Determination of protein spot abundance was performed using the DeCyder 2D Ver. 6.0 software (GE Healthcare Bio-Sciences). Spots were automatically detected. Spot editing (separation of two spots) or deleting (artifacts) was performed manually. The three CyDye–labeled forms of each spot were co-detected within each gel. Ratios between sample and internal standard abundances were calculated for each protein spot with the Differential In-gel Analysis (DIA) module. Inter-gel variability was corrected by matching and normalization of the internal standard spot maps by the Biological Variance Analysis (BVA) module of the DeCyder software and incorrectly matched spots were manually eliminated or corrected if possible. During the spot detection, the estimated number of spots was set at 4000. Protein spots that showed a statistically significant intensity in Student's *t* test were accepted as being differentially expressed between the extracts under comparison among these. Protein spots showing at least 1.25-fold changes ($p<0.05$) in intensity were selected for next steps.

Protein identification by mass spectrometry and database search

The preparative gels were stained with a SYPRO® ruby (Invitrogen/Life Technologies Corporation) and scanned with the Typhoon 9400. Protein spots that showed differences in relative fluorescence were excised from the gel using the automated spot picker (GE Healthcare Bio-Sciences). The picked gel pieces were destained with 50% CH_3CN in a 50 mM NH_4HCO_3 solution. After removal of the supernatant, cysteine residues were reduced with dithiothreitol and carbamidomethylated with iodoacetamide. In-gel trypsin digestion was performed at 37°C overnight, using sequencing grade modified trypsin (Promega, Southampton, UK) reconstituted in 100 mM NH_4HCO_3. The trypsinized gel was rinsed three times in extraction buffer (5% trifluoroacetic acid in 50% CH_3CN and 50% H_2O). The trypsinized peptides solution was dried by speed vacuum, suspended in 2% CH_3CN with 0.1% trifluoroacetic acid, and analyzed by LTQ (Fisher Scientific, Waltham, MA) liquid chromatography/linear ion trap mass spectrometry (LC-MS/MS) system. Their corresponding proteins were searched using the program Mascot database-searching software (Matrix Science, London, UK), which accesses protein identification by matching mass spectroscopy data with the protein databases NCBI (http://www.ncbi.nlm.nih.gov) and UniProt (http://www.uniprot.org/uniprot). Identification criteria included a Mascot score >45 (selected based on a corrected *p*-value <0.05).