

Chronic Treatment of Methimazole Alters Expression of Serotonin Receptors in Raphe Nuclei and Limbic System

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ABSTRACT

Thyroid hormones play an essential and critical role for metabolism, growth, and tissue differentiation in chordate. Thyroid dysfunction such as hypothyroidism is a frequent disease in adults, leading to neurological symptoms and emotional disease including depression. The interaction between thyroid hormones and serotonin of central nervous system may account for it. The present study was aimed to determine the expression level of serotonin receptors including 5HT_{1A}, 5HT_{2A}, 5HT_{2C}, 5HT_{3A}, and serotonin transporter (SERT) in hippocampus, amygdala, raphe nuclei, and medial prefrontal cortex in the rat after chronic treatment of methimazole (MMI) by using real time PCR. Results showed the mRNA level of 5-HT_{1A} was increased in raphe nuclei and hippocampus of the MMI-treated animals. The mRNA of 5-HT_{2A} receptor was elevated in raphe nuclei, and the mRNA of 5-HT_{2C} receptor and SERT were increased in hippocampus. In conclusion, we found that chronic administration of MMI changes expression of serotonin receptors in the raphe nuclei and limbic system, and could be the reason due to depression.

Keywords: 5-HT_{1A} receptor, hypothyroidism, raphe nuclei, real-time PCR

Introduction

To maintain the normal physiological function and homeostasis in the body requires the functional collaboration of neural and endocrine system. Thyroid hormones, which are secreted by thyroid gland, play an essential and critical role for the regulation of metabolism, body growth, and tissue differentiation in chordate. For example, thyroid hormones participate in the proliferation and differentiation of the central nervous system in fetus (Obregon *et al.*, 1984; Vulsma *et al.*, 1989) and also in the postnatal stage (de Escobar *et al.*, 2004). At teen ages, thyroid hormones facilitate the elongation of bones. Hypothyroidism leads to delays in body growth and intellectual development, which is called cretinism in severe cases. Hypothyroidism in adult is known as myxedema, syndrome including loss of the teeth, falling off the hair, and increased bulk of body, as due to the excess of subcutaneous fat. In addition, affections of speech, movement, sensation, consciousness, and intellect are also popular symptoms of the disease.

As early as 1960s, researchers found that

patients with severe hypothyroidism showed depression-like and dementia symptoms (Whybrow *et al.*, 1969). At around 1970s, supplementary administration of thyroid hormone became the adjuvant treatment for depression patients. This could accelerate and augment the therapeutic responses to the antidepressants. Clinical studies showed co-treating of T3 and tricyclic antidepressant (TCA) to non-refractory depression patients showed faster and fine efficacy (Prange *et al.*, 1969; Wilson *et al.*, 1970). It is widely accepted that T3 can increase the successful rate of pharmacological treatments on refractory depression (Aronson *et al.*, 1996). Recently, selective serotonin reuptake inhibitors (SSRIs) became the first choice of antidepressant worldwide. Although the clinical studies regard to the efficacy of the co-treatment of SSRI and T3 did not obtain consistent results. The meta-analysis proceeds by Papakostas at 2009 showed the co-treatment of SSRIs is more effective to thyroid dysfunction patients with the complication of depression (Papakostas *et al.*, 2009). Comparatively, T4 is less to be investigated in the studies of the therapy of emotional diseases.

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Baumgartner showed supplement of T4 in a dosage slightly above physiological concentration enhanced the efficacy of antidepressant (Baumgartner, 2000). Recently, Demartini and colleagues reported 63.5% of hypothyroidism patients with depression syndrome in Italy could not recover the depressive symptoms after the therapy with thyroid hormones (Demartini *et al.*, 2010).

Serotonin so-called 5-hydroxytryptamine (5-HT), is a derivative of tryptophan, has been proven closely associated with depression (Asberg and *et al.*, 1976; Maes and Meltzer, 1995). Although antidepressant and mental stability related drugs used in clinical are different kind of chemical compounds, are found the association of changing the neurotransmission of 5-HT (Blier and de Montigny, 1994). Electroconvulsive therapy (ECT), and antidepressants including TCA, monoamine oxidase inhibitors (MAOIs), lithium can directly or indirectly increase the level of 5-HT in the body (Blier *et al.*, 1987). Otherwise, decreased level of 5-HT in the brain induced by the decreasing of tryptophan in the body led to the symptoms relapse in SSRI-responsive depression patients (Delgado *et al.*, 1994). Correlation study showed in the cerebrospinal fluid of patients with depression, the level of 5-hydroxyindoleacetic acid, the major metabolite of 5-HT, decreased in depression patients compared with normal people (Maes and Meltzer, 1995). According to the research on the clinical brain imaging, depression is associated with the available amount of 5-HT transporter. These studies showed the mechanism of depression is possibly related with the regulation of serotonin system.

Thyroid dysfunctions are commonly present in adults, leading to neurological symptoms, could change the homeostasis of neurotransmission, affecting the central and peripheral nervous system. In thyroidectomized rats, the muscles showed a reduction in acetylcholine receptor density and dihydropyridine type calcium channel still remained normal (Kragie and Smiehorowski, 1993). Hypothyroidism increased the expression of Fos, an index of neural activity, in cholinergic neurons of brain medullary dorsal vagal complex in rats (Yuan and Yang, 2005). Thyroid hormone could regulate the expression of (N-methyl-d-aspartate) NMDA receptor subunit mRNA in adult brain. The mRNA of NR1 subunit was decreased in the hippocampus of thyroidectomized rats and restored to normal

level by the administration of T3 (Lee *et al.*, 2003). In addition, the protein level of NR1 and NR2B subunit was decreased in the hippocampal CA1 area of the thyroidectomized rats. Electrophysiological results showed that the formation of both hippocampal long-term potentiation (LTP) and long-term depression (LTD) were influenced. Which can be restored by chronic nicotine treatment (Alzoubi *et al.*, 2007).

The serotonergic system is involved in the pathogenesis of depression and is also involved by thyroid hormones. Hypothyroidism increased the turnover rate of serotonin in the brainstem of adult rats (Henley *et al.*, 1991). For 5-HT receptors, hypothyroidism induced by drugs or thyroidectomy had no effect on the density of 5-HT_{1A} receptor in the brainstem. Studies on the density of 5-HT_{1A} (postsynaptic) receptors outside the brainstem yielded contradictory results (Bauer *et al.*, 2002). The possible change of 5-HT receptors after hypothyroidism deserves further investigation. The present study was aimed to determine the expression level of serotonin receptors including 5HT_{1A}, 5HT_{2A}, 5HT_{2C}, 5HT_{3A}, and serotonin transporter (SERT) in hippocampus, amygdala, raphe nuclei, and medial prefrontal cortex. Those receptors of the brain regions are well known target sites for anxiolytic and/or anxiogenic agents (Bauer *et al.*, 2002; Haenisch and Bönisch, 2011; Quesseveur *et al.*, 2012). Briefly, hypothyroidism was induced by methimazole (MMI) treatment. Brain tissues were dissected out and prepared for real-time-PCR.

Materials and Methods

Animals

Female Wistar rats at the age of five weeks old were obtained from BioLASCO Taiwan Co., Ltd. and reared in the animal facility of National Taiwan Normal University. Three to four rats were housed in one plastic cage with bedding. The housing environment was maintained at 25°C with 12h/12h light cycle and *ad libitum* for food and water. The animals were bred according to standard procedures and following guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan Normal University. All efforts were made to minimize the animal numbers, which are required to produce meaningful experimental data.

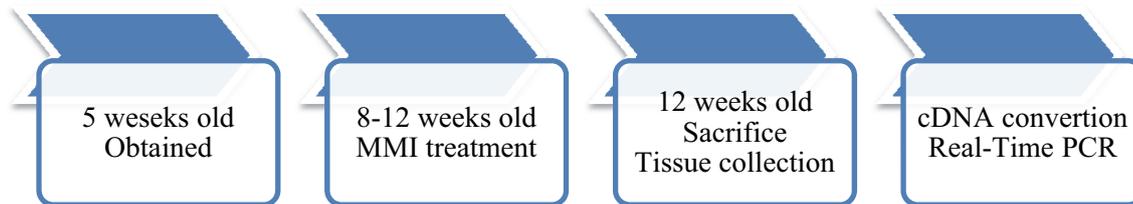


Figure 1. The flow chart of experimental procedures of this study.

The induction of hypothyroidism in rats

At eight weeks old, female Wistar rats were weighted and subjected into two groups, the control group and the methimazole (MMI) treated group. The rats of the control group drank normal water, and the MMI treated group drank the water with 0.035% MMI for a total of 4 weeks. The experimental procedure was summarized in figure-1.

Brain tissue collection and preparation

At 12 weeks old, the rats were sacrificed by decapitation, the brain tissue were removed from the skull and put into ice cold artificial cerebrospinal fluid (ACSF). The brain sections were sliced by the brain matrice for rat and the desired regions including raphe nuclei, hippocampus, amygdala, medal prefrontal cortex (MPFC) were further dissected out on the chilled stainless platform. Tissues were weighted and cooled by liquid nitrogen immediately. Tissues were then stored at -80°C deep freezer.

The extraction of total RNA

The brain tissues (less than 40 mg) were homogenized with 100 µl of Trizol and chilled on ice for 10 minutes, and then mixed with 160 µl of chloroform for 5 minutes. The homogenate was spin down at 12,000 rcf for 10 minutes. The supernatant was collected and mixed with chloroform and spin down again. Then the supernatant was collected and added with the same volume of isopropanol, put at -80°C for 1 hours, then centrifuge at 12,000 rpm for 30 minutes. The precipitated total RNA was washed with 70% DEPC treated ethanol, dried on the laminar flow, resolved in 20 µl DPEC ddH₂O. The concentration and purity of total RNA was measured by Nanodrop 1000 spectrophotometer.

cDNA conversion

One µg of trizol-extracted total RNA isolated from rat hippocampus or amygdala was mixed with 1 µl of 5 µM oligo dT(15) and 1 µl of 10 mM dNTP, heated at 65°C for 5 min and stayed on ice for 1 minute. Then 200 U of Moloney murine leukemia virus reverse transcriptase and 5X buffer, and RNase inhibitors were added. The reaction was in a volume of 20 µl and proceeded at 42°C for 1 hour. The product is diluted 100X with DEPC ddH₂O, 4 µl was taken for real time-PCR analysis.

Real time PCR

Quantitative PCR for target genes was performed using KAPA SYBR® FAST qPCR Kit on an ABI StepOnePlus Real-Time PCR system. The PCR protocol was followed with the recommend of the kit. For details, the temperature is 95°C 30 seconds, and 40 cycles of 95°C 3 seconds and 60°C 20 seconds. The sequences of primer pairs used to detect the target genes were summarized in table 1. Expression of target genes was determined using the Ct method by normalizing to the expression of GADPH.

Table 1. Summary of different primer pairs used in the real-time PCR.

Gene	Sequence
5-HT _{1A}	F 5'- GATCTCGCTCACTTGGCTCA R 5'- ACCTTCCTGACAGTCTTGCG
5-HT _{2A}	F 5'- CACCGACATGCCTCTCCATT R 5'- GGACACAGGCATGACAAGGA
5-HT _{2C}	F 5'- ATTTGTGCCCGTCTGGATT R 5'- GCTTTCGTCCCTCAGTCCAA
5-HT _{3A}	F 5'- TGGCTCTGCTGGTGATAAGC R 5'- AGTGGTTTCCCATGGCTGAG
SERT	F 5'- ATGCTACCAGAATGGCGGAG R 5'- GCCCAGGCTATGATGGTGT
GAPDH	F 5'- TGATGACATCAAGAAGGTGGTGAAG R 5'- TCCTTGAGGCCATGTAGGCCATG

Results

5-HT is produced original in neurons of midbrain raphe nuclei which is the major source of 5-HT projections in the central nervous systems. The rats were induced hypothyroidism by the treatment of MMI. The function of 5-HT neurotransmission could be influenced in MMI treated rats. By using real-time PCR to determine the level of mRNA expression and normalize with GADPH, it could reflect the changing of 5-HT receptors in different brain region. In the Raphe nuclei, the results showed the mRNA level of 5-HT_{1A} and 5-HT_{2A} receptors were increased in MMI treated rats compared with control rats (figure 2, t test, 5-HT_{1A}: $p < 0.05$; 5-HT_{2A}: $p < 0.01$). There was not significant changing in the mRNA level of 5-HT_{2C}, 5-HT_{3A}, and SERT between control and MMI treated rats.

It is well known that corticolimbic structures including prefrontal cortex (PFC), amygdala, hippocampus and nucleus accumbens (NAc), are involved in mood regulation and the stress response and also express receptors for 5-HT, both of them are extensively innervated by raphe-originating axons. In the hippocampus, the mRNA level of SERT, 5-HT_{1A} and 5-HT_{2C} receptors were increased in MMI-treated rats (figure 3, t test, 5-HT_{1A}: $p < 0.05$; 5-HT_{2C}: $p < 0.01$; SERT: $p < 0.05$). However, in the amygdala and MPFC, the mRNA level was not significant changed among different 5-HT receptors in this experiment (figure 4 and 5).

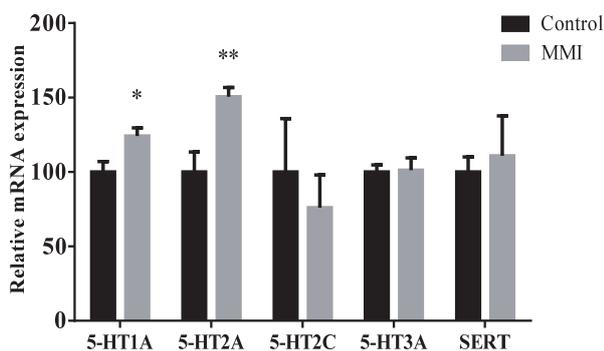


Figure 2. The mRNA level of 5-HT receptor subtypes in the raphe nuclei of the control and MMI-treated rats. The mRNA level was detected by real-time PCR and normalized with the expression of GADPH. Each value is the mean \pm SEM of 5 individual animals, which were performed in duplicate. (*: $p < 0.05$, **: $p < 0.01$)

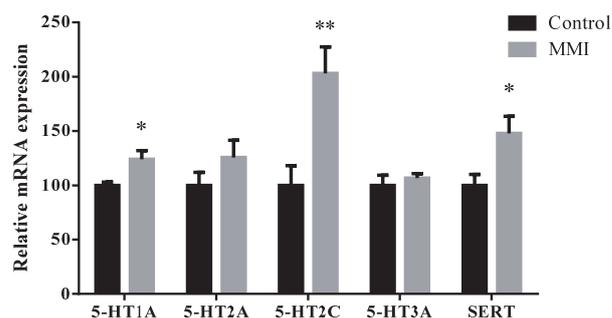


Figure 3. The mRNA level of 5-HT receptor subtypes in the hippocampus of the control and MMI-treated rats. The mRNA level was detected by real-time PCR and normalized with the expression of GADPH. Each value is the mean \pm SEM of 5 individual animals, which were performed in duplicate. (*: $p < 0.05$, **: $p < 0.01$)

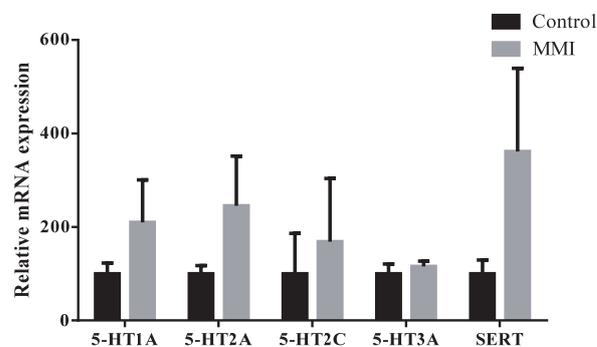


Figure 4. The mRNA level of 5-HT receptor subtypes in the amygdala of the control and MMI-treated rats. The mRNA level was detected by real-time PCR and normalized with the expression of GADPH. Each value is the mean \pm SEM of 5 individual animals, which were performed in duplicate.

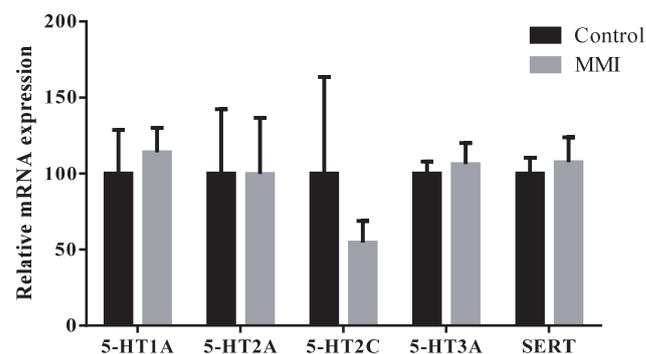


Figure 5. The mRNA level of 5-HT receptor subtypes in the MPFC of the control and MMI-treated rats. The mRNA level was detected by real-time PCR and normalized with the expression of GADPH. Each value is the mean \pm SEM of 5 individual animals, which were performed in duplicate.

Discussion

It is well known that the influence of 5-HT systems on depression, and the function of thyroid hormones was also involved in 5-HT systems and the status of depression. Previous studies showed that hypothyroid states experimentally induced in adult animals could increase the 5-HT turnover in the brainstem (Savard *et al.*, 1983; Henley *et al.*, 1991) and decrease the concentration of 5-HT in cerebral cortex (Ito *et al.*, 1977; Upadhyaya and Agrawal, 1993). In the present study, for the mimicking of the patients with hypothyroidism, rats were feed with normal food instead low-iodine food. The immunostaining of 5-HT did not change significantly in the hippocampus of MMI treated rats (data not shown). Since the sensitivity of DAB staining may not be able to reflect the small difference between the control and MMI-treated group. Therefore, we cannot exclude the possibility that the facilitation of the 5-HT turnover after MMI-treatment may confound our results. Further experiment will be required to test this possibility.

According to the evidence that the distribution of mRNA encoding the 5-HT_{1A} receptor is almost identical to that of the 5-HT_{1A} binding site (Chalmers and Watson, 1991; Burnet *et al.*, 1995). Therefore, the expression level of the mRNA could be a reliable basis for estimating the protein level of the 5-HT_{1A} receptor. It must be notified that the mRNA level of 5-HT_{1A} receptor was increased in raphe nuclei and hippocampus in the MMI-treated rats. The 5-HT_{1A} receptors are located presynaptic of the 5-HT neurons themselves and postsynaptic to 5-HT neurons in the forebrain regions. Previous electrophysiological studies evidenced 5-HT_{1A} receptor activation causes neuronal hyperpolarization, an effect mediated through the opening of G-protein-coupled potassium channels (Nicoll *et al.*, 1990; Aghajanian, 1995). The upregulation of the level of 5-HT_{1A} receptor in MMI-treated rats could repress the function of both raphe nuclei and hippocampus. To maintain the activity of hippocampus and raphe nuclei, we speculate that the increase of the excitation signaling may be required. It may explain our current finding that up-regulation of the mRNA of 5-HT_{2A} receptor was found in raphe nuclei, which may also account for the up-regulation of the mRNA of 5-HT_{2C} receptor and SERT in hippocampus. Further detailed studies are necessary to elucidate the

mechanism. Subsequent experiments such as forced swimming test and locomotor activity will be required to clarify the possible role of these phenomena and the formation of depression-like behavior in hypothyroidism. In conclusion, we suggest that the chronic effect of MMI alters expression of serotonin receptors in the raphe nuclei and limbic.

Reference

- Aghajanian G. 1995. Electrophysiology of serotonin receptor subtypes and signal transduction pathways. In: Bloom FR, Kupfer, D.J. (Eds.), *Psychopharmacology: The fourth generation of progress*. Raven. New York. 1451-1459
- Alzoubi K, Aleisa A, Alkadhi K. 2007. Adult-onset hypothyroidism facilitates and enhances LTD: reversal by chronic nicotine treatment. *Neurobiol Dis.* 26:264-272.
- Aronson R, Offman HJ, Joffe RT, Naylor CD. 1996. Triiodothyronine augmentation in the treatment of refractory depression. A meta-analysis. *Arch Gen Psychiatry.* 53:842-848.
- Asberg M, et al. 1976. "Serotonin depression": A biochemical subgroup within the affective disorders? *Science.* 191:478-480.
- Bauer M, Heinz A, Whybrow P. 2002. Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. *Mol Psychiatry.* 7.
- Baumgartner A. 2000. Thyroxine and the treatment of affective disorders: an overview of the results of basic and clinical research. *Int J Neuropsychopharmacol.* 3:149-165.
- Blier P, de Montigny C. 1994. Current advances and trends in the treatment of depression. *Trends Pharmacol Sci.* 15:220-226.
- Blier P, de Montigny C, Chaput Y. 1987. Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. *J Clin Psychopharmacol.* 7:24S-35S.
- Burnet P, Eastwood S, Lacey K, Harrison P. 1995. The distribution of 5-HT_{1A} and 5-HT_{2A} receptor mRNA in human brain. *Brain Res.* 676:157-168.
- Chalmers DT, Watson SJ. 1991. Comparative anatomical distribution of 5-HT_{1A} receptor mRNA and 5-HT_{1A} binding in rat brain - a

- combined in situ hybridisation/in vitro receptor autoradiographic study. *Brain Res.* 561:51-60.
- de Escobar GM, Obregon MJ, del Rey FE. 2004. Maternal thyroid hormones early in pregnancy and fetal brain development. *Best Pract Res Clin Endocrinol Metab.* 18:225-248.
- Delgado PL, Price LH, Miller HL, Salomon RM, Aghajanian GK, Heninger GR, Charney DS. 1994. Serotonin and the neurobiology of depression. Effects of tryptophan depletion in drug-free depressed patients. *Arch Gen Psychiatry.* 51:865-874.
- Demartini B, Masu A, Scarone S, Pontiroli AE, Gambini O. 2010. Prevalence of depression in patients affected by subclinical hypothyroidism. *Panminerva Med.* 52:277-282.
- Henley W, Chen X, Klettner C, Bellush L, Notestine M. 1991. Hypothyroidism increases serotonin turnover and sympathetic activity in the adult rat. *Can J Physiol Pharmacol.* 69:205-210.
- Haenisch B, Bönisch H. 2011. Depression and antidepressants: insights from knockout of dopamine, serotonin or noradrenaline reuptake transporters. *Pharmacol Ther.* 129:352-368.
- Ito J, Valcana T, Timiras P. 1977. Effect of hypo- and hyperthyroidism on regional monoamine metabolism in the adult rat brain. *Neuroendocrinol.* 24:55-64.
- Kragie L, Smiehorowski R. 1993. Measures of skeletal muscle calcium channels and acetylcholine receptors in thyroidectomized rats. *Endocr Res.* 19:207-219.
- Lee P, Brady D, Koenig J. 2003. Thyroid hormone regulation of N-methyl-D-aspartic acid receptor subunit mRNA expression in adult brain. *J Neuroendocrinol.* 15:87-92.
- Maes M, Meltzer H. 1995. The serotonin hypothesis of major depression. In: Bloom FR, Kupfer, D.J. (Eds.), *Psychopharmacology: The fourth generation of progress.* Raven. New York. 933-934
- Nicoll RA, Malenka RC, Kauer JA. 1990. Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiol Rev.* 70:513-565.
- Obregon MJ, Mallol J, Pastor R, Escobar GMD, Rey F. 1984. L-Thyroxine and 3,5,3'-triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. *Endocrinol.* 114:305-307.
- Papakostas GI, Cooper-Kazaz R, Appelhof BC, Posternak MA, Johnson DP, Klibanski A, Lerer B, Fava M. 2009. Simultaneous initiation (coinitiation) of pharmacotherapy with triiodothyronine and a selective serotonin reuptake inhibitor for major depressive disorder: a quantitative synthesis of double-blind studies. *Int Clin Psychopharmacol.* 24:19-25.
- Prange AJ, Jr., Wilson IC, Rabon AM, Lipton MA. 1969. Enhancement of imipramine antidepressant activity by thyroid hormone. *Am J Psychiatry.* 126:457-469.
- Quesseveur G, Nguyen H, Gardier A, Guiard B. 2012. 5-HT₂ ligands in the treatment of anxiety and depression. Expert opinion on investigational drugs. 21:1701-1725.
- Savard P, Merand Y, Di Paolo T, Dupont A. 1983. Effects of thyroid state on serotonin, 5-hydroxyindoleacetic acid and substance P contents in discrete brain nuclei of adult rats. *Neuroscience.* 10:1399-1404.
- Upadhyaya L, Agrawal J. 1993. Effect of L-thyroxine and carbimazole on brain biogenic amines and amino acids in rats. *Endocr Res.* 19:87-99.
- Vulsma T, Gons MH, de Vijlder JJ. 1989. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. *N Engl J Med.* 321:13-16.
- Whybrow PC, Prange AJ, Jr., Treadway CR. 1969. Mental changes accompanying thyroid gland dysfunction. A reappraisal using objective psychological measurement. *Arch Gen Psychiatry.* 20:48-63.
- Wilson IC, Prange AJ, Jr., McClane TK, Rabon AM, Lipton MA. 1970. Thyroid-hormone enhancement of imipramine in nonretarded depressions. *N Engl J Med.* 282:1063-1067.
- Yuan PQ, Yang H. 2005. Hypothyroidism increases Fos immunoreactivity in cholinergic neurons of brain medullary dorsal vagal complex in rats. *Am J Physiol Endocrinol Metabol.* 289:E892-E899.

長期處理 methimazole 對縫核及邊緣系統中血清素受器之影響

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摘 要

甲狀腺素為調節生理代謝作用的重要激素，先前動物研究也顯示，成年大鼠甲狀腺機能減退，可能會影響大腦中血清素系統的功能並誘發憂鬱行為的表現。本實驗利用長期在飲水中添加 methimazole，來抑制甲狀腺素的功能，並以 real-time PCR 來觀察大鼠多個腦區(包括縫核、海馬迴、杏仁核及中側前額葉內，相關血清素受體表現量的變化。結果顯示經 MMI 處理之大鼠腦縫核及海馬迴內，5-HT_{1A} 型受器的表現量均有增加的情況。此外，5-HT_{2A} 型受器在中縫核中表現量增加，而 5-HT_{2C} 型受器及血清素轉運蛋白(SERT)在海馬迴中表現量增加。這些受器的表現量改變，推測可能與維持該腦區的活性有關，並可能因此導致憂鬱症的產生。

關鍵詞：5-HT_{1A} 型受器、甲狀腺機能減退、縫核、real-time PCR

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