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Abstract

Statins have been widely used for the treatment of a variety of medical conditions including psychoneurological disorders beyond their original use in lowering cholesterol. Histamine receptors play an important role in the regulation of neural activity, however, it is unknown whether statins act on histamine receptors, particularly for their neural regulatory effects. This study examined the effects of simvastatin and 6-hydroxydopamine (6-OHDA) lesions on histamine H1 receptors using [³H] pyrilamine binding autoradiography. Compared to the saline group, simvastatin (1 mg/kg/day) significantly decreased H1 receptor bindings in the primary motor cortex (M1), ventromedial hypothalamic nucleus (VMH), caudate putamen (CPu), accumbens core (AcbC) and prefrontal cortex (Pfc) (all $p < 0.05$); however 10 mg/kg/day simvastatin increased H1 receptor density only in the medial amygdaloid nucleus (Mep) ($p < 0.05$), but had no significant effect in other regions examined. The 6-OHDA lesion did not alter H1 receptor binding density in most brain areas, except a trend decrease in the hippocampus ($p = 0.07$) and a trend increase in the cingulate cortex ($p = 0.06$). These results suggested that simvastatin has different effects on the H1 receptors in different rat brain regions depending on the doses. Therefore, simvastatin can modulate histaminergic neurotransmission in the brain, and support the role of H1 receptors in psychoneurological disorders.

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Effects of simvastatin and 6-hydroxydopamine on histaminergic H1 receptor binding density in rat brains

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Abstract

Statins have been widely used for the treatment of a variety of medical conditions including psychoneurological disorders beyond their original use in lowering cholesterol. Histamine receptors play an important role in the regulation of neural activity, however, it is unknown whether statins act on histamine receptors, particularly for their neural regulatory effects. This study examined the effects of simvastatin and 6-hydroxydopamine (6-OHDA) lesions on histamine H1 receptors using [³H] pyrilamine binding autoradiography. Compared to the saline group, simvastatin (1 mg/kg/day) significantly decreased H1 receptor bindings in the primary motor cortex (M1), ventromedial hypothalamic nucleus (VMH), caudate putamen (CPu), accumbens core (AcbC) and prefrontal cortex (Pfc) (all $p < 0.05$); however 10 mg/kg/day simvastatin increased H1 receptor density only in the medial amygdaloid nucleus (Mep) ($p < 0.05$), but had no significant effect in other regions examined. The 6-OHDA lesion did not alter H1 receptor binding density in most brain areas, except a trend decrease in the hippocampus ($p = 0.07$) and a trend increase in the cingulate cortex ($p = 0.06$). These results suggested that simvastatin has different effects on the H1 receptors in different rat brain regions depending on the doses. Therefore, simvastatin can modulate histaminergic neurotransmission in the brain, and support the role of H1 receptors in psychoneurological disorders.

Key words: histamine, receptor, simvastatin, 6-hydroxydopamine

Abbreviations:

6-OHDA, 6- hydroxydopamine; AcbC, accumbens core; AN, anorexia nervosa; CPu, caudate putamen; GPCR, G protein coupled receptors; M1, primary motor cortex; Mep, medial posterodorsal nuclei of posterior amygdala; MFB, medial forebrain bundle; PBS, phosphate saline buffer; Pfc, prefrontal cortex; TH, Tyrosine hydroxylase; SN, substantia nigra; VMH, ventromedial hypothalamic nucleus; VTA, ventral tegmental area.

Introduction

Belonging to the family of G protein coupled receptors (GPCR), histamine H1, 2 and 3 receptors are abundantly distributed in the brain while H4 receptors are mainly expressed in the peripheral tissues (De Esch et al. 2005). As one of the main histamine receptors, H1 receptors are found in the thalamus, hypothalamus, brainstem nuclei, amygdala, hippocampus, and prefrontal cortex (Martinez-Mir et al. 1990). H1 receptors are associated with an intracellular G-protein (G_q) that activates the phospholipase C, and diacylglycerol (DAG)-sensitive activation of protein kinase C (PKC), phosphatidylinositol (PIP2) signaling pathways. It has been shown that H1 receptors are involved in many important brain functions, including working memory, feeding rhythms, energy metabolism, sleeping, mood, pain and inflammation (Haas et al. 2008). Various neuropsychological disorders may lead to changes in central H1 receptors in different brain regions. The higher expression of H1 receptor binding in anorexia nervosa (AN) patients was observed in the limbic system including the amygdala, hippocampus, medial prefrontal cortex, orbitofrontal cortex and temporal cortex (Yoshizawa et al. 2009). Iwabuchi et al (2005) found that the binding densities of H1 receptors in the frontal, prefrontal and the cingulate cortices were significantly decreased in schizophrenic patients compared to the control subjects (Iwabuchi et al. 2005). On the contrary, an elevation of H1 receptor binding was detected in the foci of epileptic patients with complex partial seizures (Iinuma et al. 1993). Taken together, these findings strongly suggest that H1 receptors play important roles in some neurological dysfunctions.

Statins, the inhibitor of rate-limiting enzyme-hydroxymethylglutaryl-coenzyme reductase, have been widely used for lowering cholesterol. In addition, increasing evidence showed that statins have potential medical applications in various

diseases such as peripheral arterial disease, stroke, traumatic brain injury, and the end-stage of renal disease, diabetes mellitus, multiple sclerosis, and Alzheimer's disease. Recent studies showed that statins exhibited profoundly neuroprotective effects on dopaminergic neurodegeneration (Ghosh et al. 2009; Hernández-Romero et al. 2008; Wang 2010). To our knowledge, there is very little information regarding the relationship between statins, 6-hydroxydopamine (6-OHDA), and histaminergic H1 receptors. One study by Schuster (2008) found that lovastatin obviously reduced the severity of abnormal involuntary movements in 6-OHDA lesioned rats (Schuster 2008). Ryu et al. (1995) indicated that unilateral injection of 6-OHDA into the striatum resulted in up-regulation of H3 receptor binding sites in the dorsomedial and dorsolateral regions of the striatum and substantia nigra in rats (Ryu et al. 1995). Our previous studies and one by Selley have reported that high doses of simvastatin pronouncedly increased dopamine D1/D2 and NMDA receptors in the prefrontal cortex while also altering dopamine content in various brain regions (Selley 2005; Wang et al. 2005a; Wang et al. 2009). Considering dopamine receptors and histaminergic receptors are all GPCR and the interactions between the histaminergic and dopaminergic systems are well documented (Anichtchik et al. 2001; Anichtchik et al. 2000; Nowak et al. 2006), we considered it reasonable to propose that chronic treatment with simvastatin may affect the expression of H1 receptors in the rat brain.

In this study, the primary purpose was to explore the effects of simvastatin and 6-OHDA, a neurotoxin for dopaminergic neurons used for experimental models of Parkinson's disease, on H1 receptors in various rat brain regions. To the best of our knowledge, the influence of simvastatin on the H1 receptor binding site in the rat brain has not been systematically investigated. To address this issue, we used [³H] pyrilamine binding autoradiography, a histaminergic antagonist with high affinity for

the H1 receptors, to study histamine H1 receptors in responses to simvastatin or 6-OHDA-induced neurotoxicity treatment across a wide range of brain structures.

Materials and methods

Animals and drug treatments

Forty eight male Sprague-Dawley rats (230–250 g) were obtained from the Animal Resources Centre (Perth, Western Australia, Australia) and housed in environmentally controlled conditions (22°C, 12 h light-dark cycle with light cycle from 0600 to 1800 h and dark cycle from 1800 to 0600 h) with *ad libitum* access to standard laboratory chow and water. Rats were allowed 1 week to adapt to their new environment before experiments began. They were randomised for receiving vehicle injection (saline) or for 6-OHDA-induced Parkinsonian treatment. (One rat from the latter group died during surgery). Before surgery, six to eight rats from each group were pre-treated with 1 mg/kg/day simvastatin, or 10 mg/kg/day simvastatin, or saline for 5 days, and the same treatment for each group was continued for 3 weeks after surgery. Therefore, there were six groups: (1) 6-OHDA lesion + 1 mg/kg/day simvastatin, (2) 6-OHDA lesion + 10 mg/kg/day simvastatin, (3) 6-OHDA lesion + saline, (4) non-lesion (vehicle) + 1 mg/kg/day simvastatin, (5) non-lesion (vehicle) + 10 mg/kg/day simvastatin, and (6) non-lesion (vehicle) + saline. After three weeks of simvastatin treatment, rats from each group were sacrificed, and brains were removed and frozen in liquid nitrogen to examine histamine H1 receptor binding. This study was approved by the University of Wollongong Animal Ethics Committee and all animal experiments were conducted in compliance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23)* revised 1996 guidelines and National Health and Medical Research Council

(NHMRC) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (2004).

6-OHDA lesioned Parkinsonian rats

6-OHDA lesioned Parkinsonian rats were prepared as described in our previous works (Li et al. 2010; Wang et al. 2005b). Briefly, male Sprague–Dawley rats were anaesthetised with 75 mg/kg ketamine and 10 mg/kg xylazine (Troy laboratories Pty, Ltd, Australia). Lesions were made by injecting 6-OHDA unilaterally (4 μ l of 8 μ g/ μ l, 0.8 μ l/min) dissolved in saline containing 0.2 mg/ml ascorbic acid (Sigma-Aldrich, St Louis, MO) into the MFB at the Bregma level AP -4.4 mm, ML -1.4 mm and DV -7.8 mm (Paxinos and Watson 1997; Wang et al. 2005b)

Histology and [3 H] pyrilamine binding autoradiography

Three weeks later, rats were sacrificed by rapid CO₂ asphyxiation. In order to minimise the impact of circadian variation on binding density, the rats were sacrificed between 0700 and 0900 h, and the brains were immediately removed and frozen in liquid nitrogen. Coronal brain sections (14 μ m) were cut at -17° C with a cryotome (Clinicut cryostat; Bright Instruments), and thaw-mounted onto poly-L-lysine coated microscope slides (PolysineTM, Menzel GmbH & Co, KG) (Wang and Huang 2008). [3 H] pyrilamine autoradiography was performed following procedures as described in previous work from our laboratories and others' (Han et al. 2008; Jin and Panula 2005; Palacios et al. 1981; Ryu et al. 1995). Sections were incubated for 120 min at room temperature in 50 nM sodium potassium phosphate buffer containing 10 nM [3 H] pyrilamine (specific activity, 25.8 Ci/mmol; Perkin Elmer, Boston, MA). Non-

specific binding was determined by addition of 10 μ M triprolidine to the sections. Sections were washed in 4°C buffer (4×2 min), dipped in distilled water and dried.

Tyrosine hydroxylase immunohistochemistry staining

Tyrosine hydroxylase staining was performed as described in previous studies (Li et al. 2010; Yuan et al. 2005). Briefly, the brain sections were cut at 14 μ m and mounted on the poly-L-lysine slides as described above. Endogenous peroxidase was quenched with 0.3% H_2O_2 (30 min). The sections were washed with distilled water (3×5 min) and placed in citrate buffer 0.1 M pH 5.8 (20 min at 60–80 °C). They were then washed in distilled water followed by phosphate saline buffer (PBS) for 5 min. Non-specific binding was blocked with 1.5% normal goat serum (Vectastain rabbit IgG ABC kit) for 60 min. This was followed by application of TH primary antibody (rabbit polyclonal anti-tyrosine hydroxylase, Millipore Corporation, AB152) diluted to 1:500 in blocking solution and incubated overnight at 4 °C. After washing in PBS (3×5 min), the sections were incubated with a 1:200 dilution of the biotinylated anti-rabbit secondary antibody (Vectastain rabbit IgG ABC kit) for 60 min followed by washes with PBS (3×5 min). The horseradish peroxidase conjugate ABC (Vectastain rabbit IgG ABC kit) was applied for 60 min followed by PBS rinses (3×5 min). 3,3'-Diaminobenzidine (Vector Labs), mixed with distilled water, buffer pH 7.5, H_2O_2 , and Nickel stock (DAB, Vector SK-4100), was applied until staining was optimal as determined by light microscopy. The sections were then washed with tap water, dehydrated, cleared with xylene and coverslipped using DPX mountant for histology (Sigma 44581). After TH-staining, the image of the sections from the striatum was digitalized with a camera (Nikon D80, Nikon Japan), connected to an Olympus microscope and a computer. Density in the striatum was measured

using the NIH ImageJ Analysis software.

Quantification and statistical analysis

Quantification of binding sites was performed on a high-resolution Beta Imager (BioSpace, Paris, France) according to our previous study (Deng and Huang 2006; Huang et al. 2008; Wang 2008). In brief, sections were placed inside the detection chamber of the Beta Imager and scanned for 3.5 h at a high-resolution setting. The levels of bound radioactivity in the brain sections were directly determined by counting the number of β -particles emerging from the tissue sections, which was followed by analysis of activity in the regions of interest using the Beta Vision Plus program (BioSpace). The radioligand binding signal was expressed in counts per minute per square millimetre (cpm/mm²) and a series of sections with known quantities of ligands was used as standards in all scans, allowing the measurement of radioligand binding signals to be converted into nCi [³H] ligand per mg tissue equivalent. The average data from both hemispheres were used for further analysis. The specific binding values were obtained by subtracting non-specific binding values from the total binding values. Brain regions were identified with reference to a standard rat brain atlas (Paxinos and Watson 1997).

The data were analysed statistically using the SPSS 17.0 program (SPSS, Chicago, IL, USA). Histamine H1 receptor binding density for each brain area was analysed by two-way ANOVA (simvastatin treatment and 6-OHDA lesion) followed by a *post-hoc* Tukey–Kramer–HSD test. TH-immunoreactive data were analysed by Student *t*-test. Data were expressed as mean \pm SEM. *P* values less than 0.05 were regarded as statistically significant.

Results

Effects of 6-OHDA lesion on TH immunoreactivity in the caudate putamen

Coronal section (Fig. 1) throughout the striatum indicated the extent of nigrostriatal denervation induced by injecting the neurotoxin 6-OHDA into the right MFB, as seen by tyrosine hydroxylase immunoreactivity. The right side of the photo (Fig. 1A) shows the dopamine-depleted striatum side characterized by a significant absence of TH immunoreactive fibres. The density of TH-positive fibres was expressed as a mean percentage \pm SEM relative to the intact side, in which the dense TH-immunoreactive fibres were found throughout the striatum. Compared to the intact side, around 80% reduction in the density of TH-positive fibres in the striatum was observed in 6-OHDA-induced Parkinsonian rats (Student *t*-test: $t=15.348$, *** $p<0.001$, Fig. 1B).

Effects of simvastatin and 6-OHDA lesion on [³H] pyrillamine binding

Specific [³H] pyrillamine binding was observed in most brain regions examined, although there were regional variations in the densities (Fig. 2). Non-specific binding was observed to be less than 5%. Among these brain regions, high levels of [³H] pyrillamine binding density were observed in the ventromedial hypothalamic nucleus (VMH) and hippocampus. Lower levels of [³H] pyrillamine binding were observed in the accumbens core (AcbC), caudate putamen (CPu) and substantia nigra (SN).

Two-way ANOVA revealed that simvastatin had a significant effect on [³H] pyrillamine binding in the VMH, Mep, AcbC, CPu, M1 and Pfc. However 6-OHDA had no significant effect in these regions and there was also no significant interaction between the simvastatin and 6-OHDA. Effects of simvastatin treatment (1 mg/kg/day) on [³H] pyrillamine binding were observed. *Post-hoc* analysis revealed that

simvastatin at 1 mg/kg/day significantly decreased [³H] pyriline binding density in the VMH (21.79%, p=0.014, Fig 4), AcbC (37.98%, p=0.034, Fig 4), CPu (27.44%, p=0.030, Figs 3 and 4), primary motor cortex (31.60%, p=0.002, Fig 3 and 4) and the prefrontal cortex (42.34%, p=0.034, Fig 3 and 4) compared with the controls. On the other hand, simvastatin at 10 mg/kg/day significantly increased [³H] pyriline binding density in the Mep (21.93%, p=0.012, Fig 4), although no significant change was observed in other regions at this treatment dosage. No significant effects of simvastatin were observed in the hippocampus, SN and Cg.

6-OHDA lesions showed a tendency to decrease [³H] pyriline binding density in the hippocampus (11.11%, p = 0.07; Table 1), and to increase [³H] pyriline binding density in the cingulate cortex (11.39%, p = 0.06; Table 1) compared to the controls. Two-way ANOVA showed that there was no significant interaction between simvastatin administration and 6-OHDA lesion in all tested brain regions.

Discussion

The present study aimed to investigate the alterations of [³H] pyriline binding density in various rat brain regions after simvastatin and/or 6-OHDA treatment. Specific [³H] pyriline binding was observed in most brain regions although there were regional variations in density. It has been reported that H1 receptors displayed high expression level and distribution pattern in the deep cortical layers and midbrain (Jin and Panula 2005). This current study showed that H1 receptors were dominant in some limbic regions such as the hippocampus, medial posterodorsal amygdala, CPu and SN; some cortical regions like the cingulate, piriform and primary motor cortex; and some midbrain regions including the ventral

tegmental area and ventromedial hypothalamus (Tab 1). This observation is consistent with the distribution of H1 receptors in the brain (Han et al. 2008; Iwabuchi et al. 2005; Jin and Panula 2005).

It has been shown that there is a close and extensive interaction between dopaminergic and histaminergic systems (Anichtchik et al. 2000). In this study, a pronounced reduction of TH immunoreactivity in the caudate putamen of the 6-OHDA-lesioned side was observed, indicating an obvious dopaminergic neuronal degeneration and complete nerve terminal denervation which might lead to establishment of a successful Parkinson disease animal model (Fig 1). This study showed that 6-OHDA-induced neurotoxicity did not alter H1 receptor binding in the brain regions including the primary motor cortex, piriform cortex, medial posterodorsal amygdala, CPu, VMH, ventral tegmental area and substantia nigra, although a tendency to increase H1 receptor density in the cingulate cortex and to decrease in the hippocampus was observed. The reasons for this unchanged pattern of [³H] pyrilamine binding in the brain may be due to the following factors: 1) 6-OHDA-induced neurotoxicity in the medial forebrain bundle did not interfere with H1 receptor expression, or may not affect [³H] pyrilamine binding to the receptors; and 2) the short time frame used in our study to examine the H1 receptor might not produce an obvious alteration in [³H] pyrilamine binding. Whether H1 receptors will change after long-term treatment remains to be determined.

The low/high dose of simvastatin chosen in this work was based on our previous study (Wang et al. 2005a). In that study, we used the low/high dose of simvastatin (1 and 10 mg/kg/day) and found that simvastatin obviously increased D1/D2 receptors in the brain. In addition, we found that simvastatin at those dosages did not affect cholesterol levels in rodents, and proposed that the reason may be the

different metabolism of lipids between rodents and human beings. The results were consistent with other reports (Roglans 2002; Schoonjans 1999). Clinically, we normally treat patients with high cholesterol or triglyceride using 20-80 mg/person/day. If the patient is around 60-80 kg, the minimal human dose should be 20 mg/60-80kg/day (namely 0.25-0.33 mg/kg/day), and the maximum human dose concentration should be 80 mg/60-80 kg/day (namely 1-1.33 mg/kg/day). In view of the fact that rats have a 4-6 times faster metabolic rate than humans, the low/high dosages of simvastatin used in the present study should be relevant to that used in human beings. In this study, it was shown that compared with the vehicle group simvastatin at low dosage (1 mg/kg/day) significantly decreased H1 receptor binding in the examined brain regions including the ventromedial hypothalamus, accumbens nucleus, caudate putamen, primary motor cortex, and prefrontal cortex; however, the high dosage of simvastatin (10 mg/kg/day) only had an increased trend but not statistically significant influence on H1 receptors except in the Mep. This fact suggests that simvastatin at different dosages may differentially regulate H1 receptor binding, and low doses but not high doses of simvastatin may pharmacologically prevent [³H] pyrilamine binding to the receptors, or enhance the receptor degradation and desensitization. Furthermore, since a single concentration of [³H] pyrilamine was used in this study, we were not able to determine whether the changes observed were due to changes in K_d or B_{max}. However, consistent with previous reports (Barbara et al. 2002; Tran et al. 1978), our pre-experiment showed that 10 nM [³H] pyrilamine was a saturation concentration in our preparation. Therefore, [³H] pyrilamine binding density should reflect the binding density of H1 receptors. Following the procedures in our laboratories and others', the slides were not preincubated (Han et al. 2008; Jin and Panular 2005; Palacios et al.1981; Ryu et al. 2005). However, to our knowledge,

there is no published evidence that simvastatin could directly inhibit H1 receptor binding or cause histamine release.

Our previous study showed that simvastatin (1 mg/kg/day) significantly increased muscarinic M1/4 receptor binding in the rat brain (Wang et al. 2008); while Miyoshi (2004) showed that in Chinese hamster ovary (CHO) cells, stimulation of muscarinic M1/4 could lead to down-regulation of H1 receptors (Miyoshi 2004). Belonging to GPCRs, H1 receptor and muscarinic M1/4 receptors are co-expressed in various tissues (Miyoshi 2004). Therefore, it was reasonable to propose that the effect of simvastatin on H1 receptors could be specific and due to, for instance, interference with cell membrane integrity through interaction with muscarinic receptors. Although there is a lack of studies about the direct effect of simvastatin on histamine content in the brain, we cannot exclude the possibility that a low dose of simvastatin increases the histamine content in the brain or enhances histamine innervation, subsequently resulting in decreased H1 receptor binding. In addition, the decreased H1 receptor binding in this study following low dose simvastatin treatment provides indirect evidence for the plasticity of the histaminergic systems in the rat brain. However, the precise mechanisms underlying decreased H1 receptor binding following low doses of simvastatin remains to be determined. Our finding suggests that simvastatin-induced decrease in H1 receptor binding may play an important role in H1 receptor mediated neuropsychiatric disorders.

Several lines of evidence have shown that food intake and dietary manipulation regulates central histamine receptors (Yoshimatsu 2006). Mercer et al. (1994) showed that decreasing central histamine or blockade of H1 receptors increased food intake in rats fed the low protein diet (Mercer et al. 1994). Similarly, Haq et al. (1996) also found that the increased H1 receptors in the brain were closely

correlated to the decrease in food intake, whereas decreases of H1 receptors were associated with an increase in food intake (Haq et al. 1996). H1 receptor knockout (H1RKO) mice develop obesity accompanied by increased food intake and altered diurnal feeding patterns (Masaki et al. 2004). H1 receptors also play a key role in antipsychotic-induced weight gain and food intake (Deng et al. 2010; Kroeze et al. 2003; Matsui-Sakata et al. 2005). The H1 receptors are mainly located in postsynaptic areas and are found abundantly in the cerebral cortex and limbic areas including the hypothalamus, amygdala and hippocampal areas (Martinez-Mir et al. 1990); while some of the brain regions exert food intake and dietary regulation. In the limbic system, the ventromedial hypothalamus plays an important role in the regulation of food intake, obesity or energy metabolism, and anorectic action. One study by Magrani et al. (2004) showed that pharmacological blockade of both H1 and H2 receptors in the VMH significantly increased food intake and decreased water intake (Magrani et al. 2004). Similarly, our recent study also showed that a profound increase in body weight gain and fat deposits induced by olanzapine were associated with a reduction of H1 receptors in the ventromedial hypothalamus (Han et al. 2008). One limitation in this study was that the body weight and behaviours of rats were not monitored during the simvastatin dosing. Therefore, it is important in further studies to measure the behaviours and weight of rats, and also to investigate whether the downregulation of H1 receptors in the ventromedial hypothalamus following simvastatin treatment is correlated to body weight.

It has been shown that central H1 receptors were increased in patients with AN. One study by Yoshizawa demonstrated that using positron emission tomography examination, when compared to male subjects, female AN patients displayed higher expression of H1 receptors in the limbic system such as the amygdala, hippocampus,

prefrontal, orbitofrontal, and temporal cortex (Yoshizawa et al. 2009). The alterations in histaminergic neurotransmission and H1 receptors in patients with AN implied that modulation of the histaminergic system might be useful in the treatment of this disease. Therefore, we propose that a low dose of simvastatin might display beneficial effects in AN patients since it would enhance the patients' food intake and diet.

Conclusion

In summary, this study examined the effects of 6-OHDA or simvastatin on H1 receptor binding in the rat brain. Low doses of simvastatin but not 6-OHDA significantly decreased the levels of H1 receptor binding, predominantly in the primary motor cortex, cingulate piriform cortex, ventromedial hypothalamus, prefrontal cortex, and the caudate putamen. The changes in H1 receptor density in these brain regions following low doses of simvastatin treatment suggest that H1 receptors may have important clinical implications in neuropsychological disorders. This result may contribute, at least partially, to dietary dysfunctions such as anorexia nervosa in patients via H1 receptors. A better understanding of the roles and relationships among statins and histaminergic systems may open new perspectives for the statin family in the modulation of psycho-neurodegenerative disorders.

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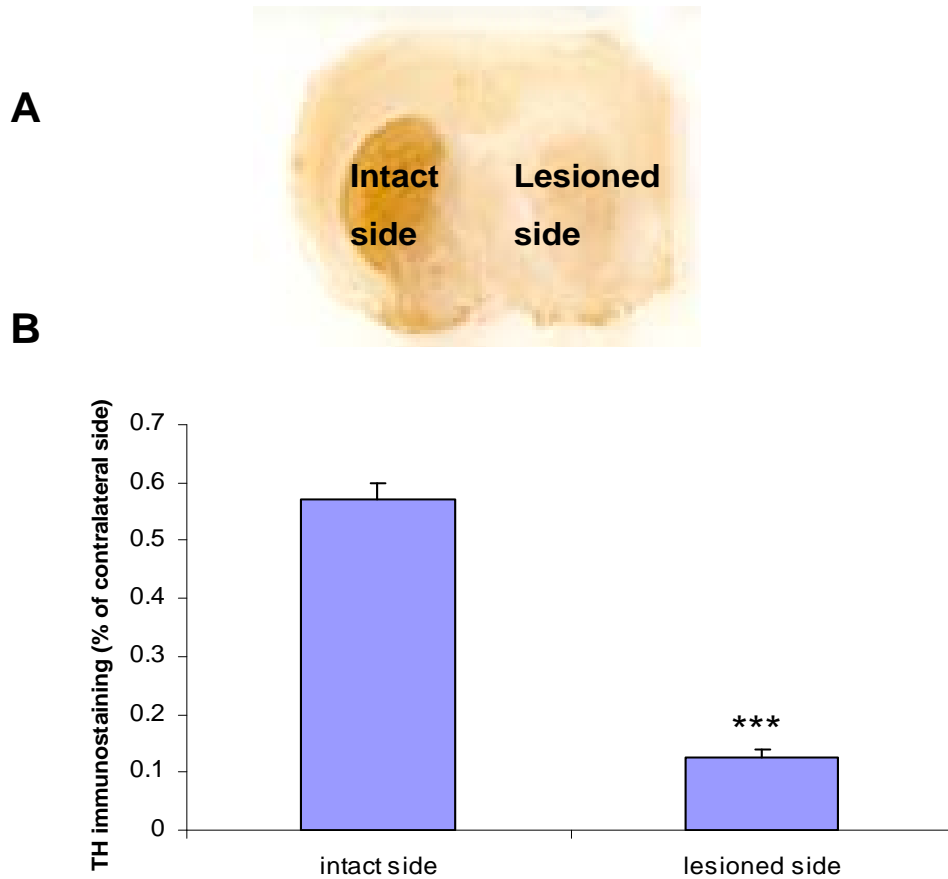


Fig 1. Effects of 6-OHDA lesion on TH immunohistochemistry staining in the caudate putamen (CPu). The right side of the photo (A) shows the dopamine-depleted striatum side characterized by a significant lack of TH immunoreactive fibres, while the left side is the intact side. Photo (B) represents the density of TH-positive fibres relative to the contralateral (intact) side (Student *t*-test: $t=18.551$, $***p < 0.001$).

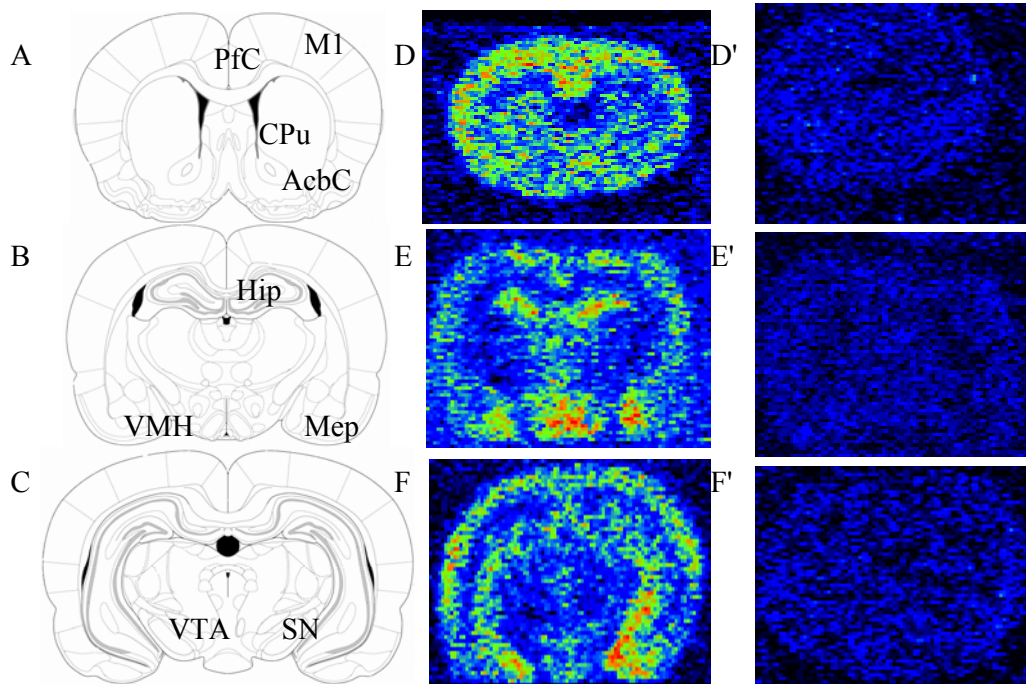


Fig 2. The maps of A, B and C are adopted from a rat brain atlas (Paxinos and Watson 1997), indicating the brain levels where the [^3H] pyrilamine binding density was measured. Autoradiographs D, E, F and D', E', F' depict the expression of [^3H] pyrilamine binding and non-specific [^3H] pyrilamine binding at different rostro-caudal coronal levels of the rat brain from the control group. Abbreviations: AcbC, accumbens nucleus; Cg, cingulate cortex; CPu, caudate putamen; Hip, hippocampus; M1, primary motor cortex; Mep, medial posterodorsal nuclei of posterior amygdala; PfC, prefrontal cortex; SN, substantia nigra; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

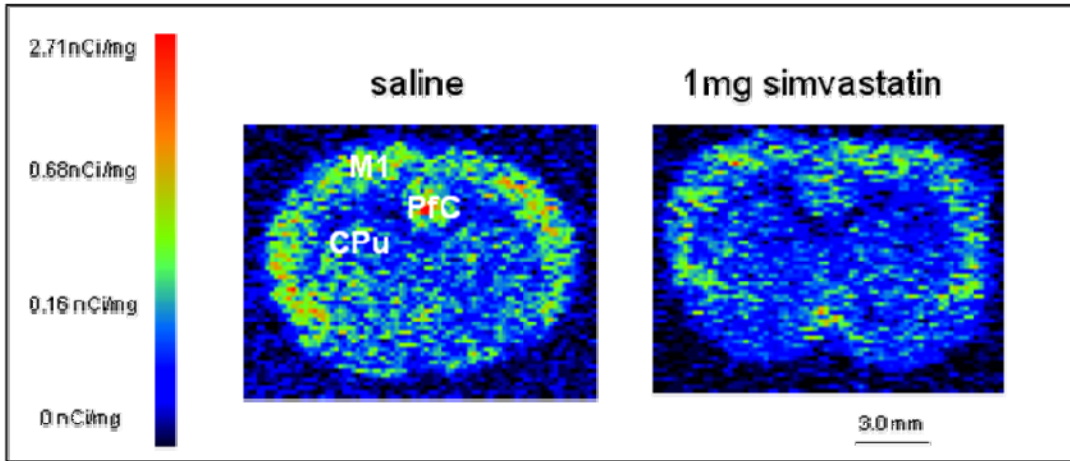


Fig 3. Typical autoradiographs depict the densities of H1 receptors in the prefrontal cortex (PfC), caudate putamen (CPu) and primary motor cortex (M1) after simvastatin (1mg/kg/day) treatment.

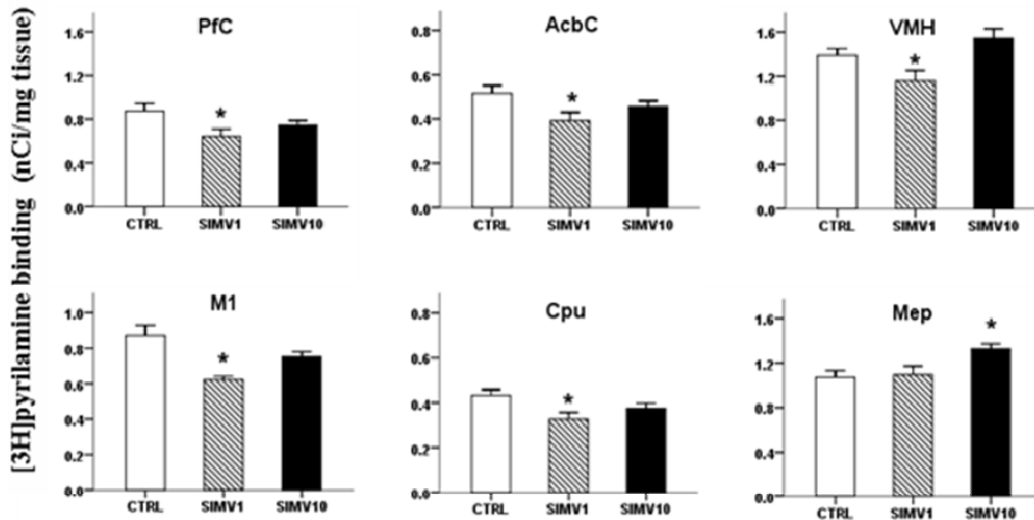


Fig 4. The effects of simvastatin administration on [³H] pyrilamine binding (nCi/mg tissue) in rat brain regions. Data shown are the mean values \pm SEM. Abbreviation: AcbC, accumbens nucleus; caudate putamen (CPu); CTRL, control; Mep, medial posterodorsal nuclei of posterior amygdala; Pfc, prefrontal cortex; M1, primary motor cortex; SIMV1, simvastatin 1mg/kg/day; SIMV10, simvastatin 10 mg/kg/day; VMH, ventromedial hypothalamus. For other abbreviations see Fig. 1. Asterisks indicate significant differences between the simvastatin treatment group and the control group (saline) ($*p < 0.05$; two-way ANOVA followed by *post-hoc* Tukey–Kramer–HSD test).

Table 1. Specific [³H] pyrilamine binding (nCi/mg tissue; mean±SEM) in different brain regions following 6-hydroxydopamine lesion and sham.

Brain region	Sham (n=6; nCi/mg)	6-OHDA (n=6; nCi/mg)	P Value
hippocampus	1.17±0.05	1.04±0.03	0.07
cingulate cortex	0.79±0.05	0.88±0.03	0.06
piriform cortex	0.77±0.05	0.75±0.06	0.72
primary motor cortex	0.78±0.04	0.76±0.03	0.71
medial posterodorsal amygdala	1.14±0.05	1.22±0.06	0.55
caudate putamen	0.39±0.02	0.37±0.02	0.94
accumbens nucleus	0.47±0.03	0.45±0.3	0.99
substantia nigra	0.52±0.04	0.57±0.04	0.30
ventral tegmental area	0.84±0.03	0.79±0.05	0.58
ventromedial hypothalamus	1.35±0.06	1.37±0.08	0.82