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Modulation of the suppressive effect of corticosterone on adult rat hippocampal cell proliferation by paroxetine

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Abstract: Objective The literature has shown that cognitive and emotional changes may occur after chronic treatment with glucocorticoids. This might be caused by the suppressive effect of glucocorticoids on hippocampal neurogenesis and cell proliferation. Paroxetine, a selective serotonin reuptake transporter, is a commonly used antidepressant for alleviation of signs and symptoms of clinical depression. It was discovered to promote hippocampal neurogenesis in the past few years and we wanted to investigate its interaction with glucocorticoid in this study. **Methods** Adult rats were given vehicle, corticosterone, paroxetine, or both corticosterone and paroxetine for 14 d. Cell proliferation in the dentate gyrus was quantified using 5-bromo-2-deoxyuridine (BrdU) immunohistochemistry. **Results** The corticosterone treatment suppressed while paroxetine treatment increased hippocampal cell proliferation. More importantly, paroxetine treatment could reverse the suppressive effect of corticosterone on hippocampal cell proliferation. **Conclusion** This may have clinic application in preventing hippocampal damage after glucocorticoid treatment.

Keywords: paroxetine; bromodeoxyuridine; corticosterone; hippocampus; cell proliferation; neurogenesis

1 Introduction

Corticosteroids are used in treatment of a wide range of medical conditions for their potent anti-inflammatory and immunosuppressive properties. However, high corticosteroid levels are frequently associated with cognitive impairment[1]. Psychosis, as well as difficulties in maintaining attention and concentration, loss of memory, and impaired logical thinking have been reported[2]. Similar findings were also reported in patients with Cushing disorder that is characterized by sustained hypercortisolemia[3].

Corticosteroid treatment in rats induces a morphological reorganization in the hippocampus, such as reversible atrophy of subfield CA3 neuronal dendritic spines[4;5] and decrease in neurogenesis in the dentate gyrus[6]. The dentate gyrus is one of the few areas exhibiting neurogenesis in the adult brain. These newly formed neurons constantly arise in the subgranular zone of dentate gyrus and migrate to the granule cell layer. They appear to actively participate in the formation of hippocampal-dependent memories[7] and are likely to play an important role in the behavioral effects of antidepressants[8].

In contrast to corticosteroids, various antidepressants have been shown to increase neurogenesis[9]. Paroxetine is a specific and potent selective serotonin reuptake inhibitor (SSRI) antidepressant commonly used in psychiatry. The purpose of the current study was to see whether the long term use of paroxetine could prevent or reverse the reduced cell proliferation which precedes the neurogenesis changes associated with corticosterone. This may lead to more effective therapeutic strategies to prevent the occurrence of psychiatry disorders during the corticosteroid treatment in the patients.

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2 Materials and methods

2.1 Animals and design of the study

Experimental procedures were carried out according to the animal ordinance set by the government of Hong Kong. Adult male Sprague Dawley rats (Laboratory Animal Centre, The University of Hong Kong) weighing 200g were housed two per cage and kept on a 12-h light-dark cycle with access to food and water *ad libitum*.

The rats were divided into 4 treatment groups ($n = 4$ per group): (A) Control group: 14-day of sesame oil and sterile water injection, as controls of corticosterone and paroxetine respectively; (B) CORT group: 14-day of corticosterone injections (40 mg/kg, subcutaneously); (C) Px group: 14-day of paroxetine injections (5 mg/kg, intraperitoneally); (D) P + C group: 14-day of both corticosterone and paroxetine injections (the same dose schedule as above). Paroxetine injections were administered twice daily at 9 a.m. and 5 p.m.

2.2 Materials

2.2.1 Administration of corticosterone Corticosterone was suspended in sesame oil (33.3 mg/mL; Sigma-Aldrich) and injected as previously described[10]. This dose is adequate to elevate blood levels of corticosterone over a 24-h period and can reduce the neurogenesis in the dentate gyrus[10,11].

2.2.2 Administration of BrdU BrdU was dissolved in 0.9% normal saline and administered intraperitoneally. All rats received six injections of BrdU (50 mg/kg) in 12-h intervals, during day 12-14 of the CORT/paroxetine/vehicle injection regime.

2.2.3 Tissue Preparation Twenty-four hours after the last injection of BrdU, the animals were injected with pentobarbital (80 mg/kg) and perfused transcardially with normal saline, followed by 4% paraformaldehyde. The adrenal glands were dissected and weighted to assess the degree of atrophy as a measurement of the efficacy of the CORT treatment. The brain was removed, post-fixed in 4% paraformaldehyde at 4 °C overnight and then prepared for coronal sections. The brains were first left in 30% (W/V) sucrose in PBS until they sank. Coronal sections were cut at 50 μm thickness through the hippocampus with a freezing microtome, put into a 12-well plastic plate in series and stored in antifreeze cryoprotectant solution at -20°C until the staining procedure.

2.2.4 Cresyl violet staining Brain sections were rinsed three times in 0.01 mol/L PBS, mounted on poly-L-lysine coated slides and air dried overnight. After a de-fat step, they were rinsed and stained in 0.1% cresyl violet solution for 3-5 min followed by rinsing in distilled water, differentiation, dehydration and coverslipping.

2.2.5 BrdU immunohistochemistry A modified protocol for BrdU staining was used to detect dividing cells. One well from every 12-well plate of stored hippocampal slices was selected. Frozen slices were slide-mounted and boiled in citric acid (pH 6.0) for 10 min, followed by PBS rinses. The brain sections were incubated in 1 mol/L HCL (37 °C, 30 min) and then boric acid buffer (pH 8.5, 10 min). After washing in PBS, sections were incubated for 30 min with hydrogen peroxide (H_2O_2) to eliminate endogenous peroxidase. After blocking with 5% normal goat serum in 0.01% Triton X-100, sections were incubated with anti-mouse BrdU (1:400, Roche) overnight at 4 °C. Sections were then incubated for 1 h with secondary antibody (1:200 biotinylated goat anti-mouse; Vector Laboratories) followed by amplification with an avidin-biotin complex (Vector Laboratories) and cells were visualized with diaminobenzidine. Chemical supplier was Sigma-Aldrich unless otherwise indicated.

2.3 Data quantification and statistical analysis The cell counting on cresyl violet stained cells was performed as described previously[10]. By delineating the granule cell layer and the hilus on four randomly chosen cresyl violet stained sections from each animal, using Neurolucida software and an Olympus BH-2 microscope with a 10 × objective and CCD-IRIS colour video camera, the number of living neurons in the CA3 and dentate gyrus of the cross-sectional areas of these regions were obtained.

The counting of BrdU-positive cells were performed as reported by Mirescu *et al*.[12]. BrdU-positive cells on every twelfth unilateral section through the whole dentate gyrus were counted at × 1 000 magnification, with the aid of *Stereo Investigator* (MicroBrightField, Williston, VT). The number of BrdU-labeled cells per dentate gyrus was then multiplied by 24 to estimate the total number of BrdU-positive cells through the dentate gyrus. The number of BrdU-positive cells in the subgranular zone in each group was described as percentage of control (vehicle).

Data were presented as mean ± SEM and were assessed by the Kruskal-Wallis one way ANOVA followed by the Mann-Whitney U-test. A probability level of $P = 0.05$ was used to determine statistical significance.

3 Results

3.1 The effect of corticosterone and paroxetine on hippocampal neurons and adrenal weight No cells with pyknotic appearance were detected by cresyl-violet staining in any treatment group. There was no significant difference in the numbers of cresyl-violet stained cells in the CA3 and dentate gyrus across groups. Corticosterone-treated rats showed decreased adrenal gland weight (Fig. 1) when compared with the control group, which indicates that the corticosterone level was elevated.

3.2 The effect of corticosterone on hippocampal cell proliferation The number of proliferating cells was identified by immunohistochemical detection of BrdU within the nuclei of dividing cells. The BrdU-positive nuclei were often

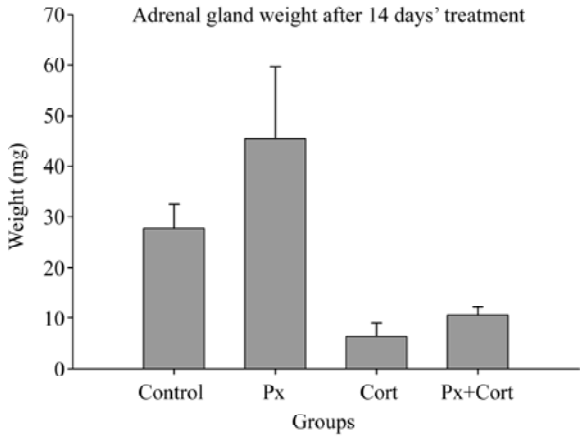

Fig.1 Weight of rat adrenal gland after 14-day treatment. Values are expressed as mean ± SD. Control: vehicle injection group; Px: paroxetine treatment group; Cort: corticosterone treatment group; Px+Cort: paroxetine and corticosterone co-treatment group.

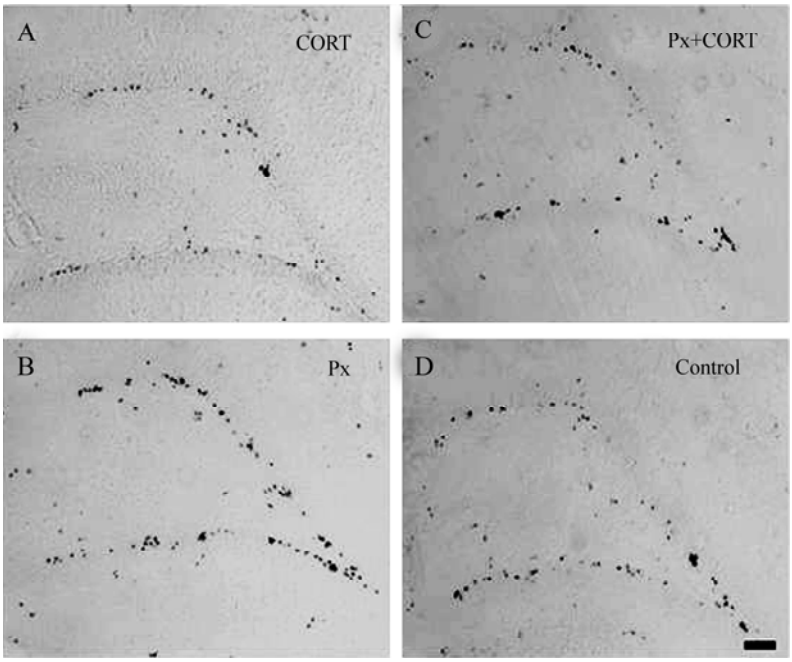

Fig. 2 Chronic corticosterone (CORT) treatment resulted in reduced numbers of BrdU-positive cells detected in the dentate gyrus (A) compared with that seen in vehicle-injected rats (D). Chronic paroxetine (Px) resulted in increased numbers of BrdU-positive cells in the granule cell layer of both vehicle-treated (B) and CORT-treated (C) and rats. Scale bar, 10 μm.

clustered in the subgranular layer and exhibited irregular shapes. Compared with the vehicle group (2479±46.8 BrdU-positive cells), corticosterone markedly reduced the number of BrdU-labeled cells (1749±70.7 BrdU-positive cells; 70.6% of control) in the dentate gyrus (Fig. 2, 3).

3.3 The effect of paroxetine on hippocampal cell proliferation Chronic treatment with paroxetine increased BrdU-labeled cells (3238±64.2 cells; 130.6% of control) in the granule cell layer compared with the groups which received CORT or vehicle alone (Fig. 2, 3). The number of BrdU-positive cells in the combined CORT and paroxetine treatment group was not significantly different from the vehicle-treated controls (2279±83.4 BrdU-positive cells; 91.9% of control, Fig. 3).

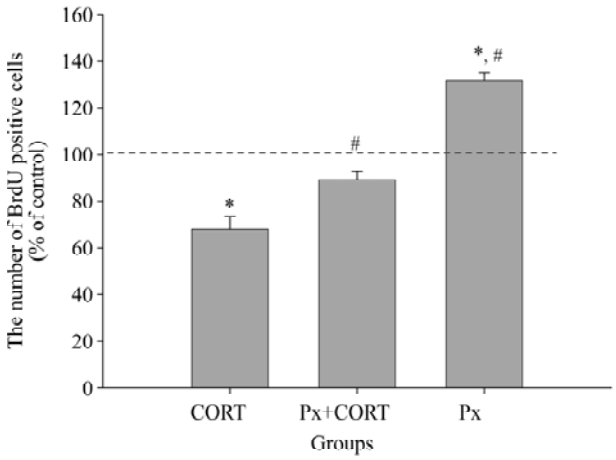

Fig. 3 Effects of corticosterone (CORT) and paroxetine (Px) alone and/or combined on the number of BrdU-labeled cells in the granule cell layer. BrdU-labeled cells in the subgranular zone were counted, and the results were normalized as percentages of the value of the control group. The data were expressed as mean ± SEM. One hundred percent of control = 2470 ± 31.56 BrdU-labelled cells. CORT = 67.91 ± 5.95 percent of control; Px + CORT= 89.25 ± 3. 66 percent; Px = 131.81 ± 3.40 percent. * $P < 0.001$ *vs* control (vehicle-treated rats), # $P < 0.001$ *vs* CORT-treated rats (one-way ANOVA).

4 Discussion

The purpose of this experiment was to determine paroxetine's effect on hippocampal cell proliferation and analyze the interaction between corticosterone and paroxetine. Using BrdU to label newly born cells, we found that the number of BrdU-positive cells increased by 31 % after treatment with paroxetine compared with the control group.

Paroxetine belongs to the group of SSRI antidepressant which can increase serotonin concentration in the synapses. SSRIs can interact with serotonergic pathways and stimulate cell proliferation. Activation of the serotonin-1A (5-HT_{1A}) receptor can induce neurogenesis[13] and chronic administration of selective 5-HT_{1A} and 5-HT_{2C} receptor agonists produces an increase in the number of newly formed neurons in the dentate gyrus and/or subventricular zone, underscoring the beneficial effects of serotonin on adult neurogenesis[14]. Paroxetine, by inhibiting serotonin reuptake, would be expected to enhance serotonin availability to these serotonergic receptors. This contrasts with corticosteroids which have been reported to reduce 5-HT_{1A} receptor mRNA expression[15] or the response of 5-HT_{1A} receptors in the hippocampus[16]. Previous research has found that the effect of corticosteroids on cell proliferation is dependent on *N*-methyl-D-aspartate (NMDA) receptors, since inhibition of NMDA receptors prevented the corticosteroid-mediated inhibition of cell proliferation[17]. The interaction of paroxetine and NMDA receptor is not yet known. It is likely that paroxetine restores cell proliferation to control levels in the corticosterone-treated rats through serotonergic pathways but the confirmation of this view requires further experimentation.

The mechanisms responsible for the antidepressant effect on cell proliferation and neurogenesis are not completely known but are likely to involve more than one pathway. Previous reports support a role for the cAMP-CREB (cyclic adenosine monophosphate – cAMP response element binding protein) cascade and brain derived neurotrophic factor (BDNF) in antidepressant effects on neurogenesis[18]. BDNF acts via trkB receptors and activates the Ras-Raf-MEK-ERK cascade (mitogen-activated protein kinase) which is critical for cell survival. It phosphorylates CREB and confers cellular survival by increasing the expression of anti-apoptotic protein Bcl-2[19]. In additional, the CREB pathway may regulate the survival, and possibly the differentiation and function of newborn neurons[20].

In summary, our experiments show that paroxetine reverses the decrease in cell proliferation caused by corticosterone in the dentate gyrus of the adult hippocampus. Reduced hippocampal volume was associated with a wide range of neuropsychiatric disorders, such as major depression, Alzheimer's disease, epilepsy, personality disorder and Parkinson's disease[21]. Although the association of major depression and decreased hippocampal volume suggests a possible causal relation, it was found that antidepressant treatment did not alter hippocampal volume[22]. Such findings suggest that decreased hippocampal volume may not be the cause of depression. The phenomenon may be rather an associated event or consequence of depressive disorder. The result of this study may lead to new strategies for neuroprotection against the adverse central nervous system effects of high corticosterone levels, which are present in the patients suffering from a variety of psychiatric disorders or in the patients receiving high dose corticosteroid treatment for conditions such as severe acute respiratory syndrome (SARS)[23]. Further studies will be necessary to dissect the interaction between corticosteroids and antidepressant drugs at the cellular level and the specific pathways or mechanisms involved.

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