Original Research Article

Anti-hyperprolactinemia mechanism of Radix bupleuri extract in rats

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Abstract

Purpose: To determine the mechanism underlying the anti-hyperprolactinemia effects of Radix bupleuri extract (RBE) in rats.
Methods: Rats were divided into six groups (n=10 each group): healthy controls, untreated hyperprolactinemic rats, hyperprolactinemic rats treated with bromocriptine (0.6 mg/kg), and hyperprolactinemic rats treated with RBE (4.8, 9.6, or 19.2 g/kg). After 30 days, hypothalamic protein levels of dopamine D2 receptor, protein kinase A (PKA), and cyclic adenosine monophosphate (cAMP) were determined.
Results: Dopamine D2 receptor levels were lower in untreated hyperprolactinemic rats than in healthy controls ($p < 0.01$), but this decrease was attenuated by RBE ($p < 0.05$). Elevated PKA levels in untreated hyperprolactinemic rats (0.61 ± 0.04 μg/ml, $p < 0.01$) were decreased by RBE (4.8 g/kg, 0.42 ± 0.03 μg/ml, $p < 0.05$; 9.6 g/kg, 0.33 ± 0.02 μg/ml, $p < 0.01$; 19.2 g/kg, 0.27 ± 0.03 μg/ml, $p < 0.01$). Similarly, elevated cAMP levels in hyperprolactinemic rats (2.4 ± 0.4 ng/ml) were decreased by RBE (4.8 g/kg, 1.8 ± 0.3 ng/ml, $p < 0.05$; 9.6 g/kg, 1.5 ± 0.3 ng/ml, $p < 0.01$; 19.2 g/kg, 1.2 ± 0.2 ng/ml, $p < 0.01$).
Conclusions: RBE anti-hyperprolactinemia activity is mediated by dopamine D2 receptor signaling via the cAMP/PKA pathway.

Keywords: Hyperprolactinemia, Radix bupleuri, Dopamine D2 receptor, cAMP/PKA

INTRODUCTION

Hyperprolactinemia is a heterogeneous disorder characterized by elevated blood prolactin level. This disorder of the hypothalamic-pituitary axis can have a physiological cause (e.g., pregnancy), pathological cause (e.g., tumor), or unknown cause (i.e., idiopathic hyperprolactinemia). In different patients, similarly elevated prolactin levels can result in severe clinical manifestations or no symptoms at all [1]. Hyperprolactinemia is more common in women than in men. Its prevalence in an unselected adult population is 0.4 % but has been reported as 5 % in women visiting a family planning clinic, 9 % in women with adult-onset amenorrhea, and 17 % in women with polycystic ovary syndrome [2,3]. The two most commonly prescribed drugs for the treatment of hyperprolactinemia are bromocriptine and cabergoline. These medications are dopamine receptor agonists with similar characteristics and adverse effects (e.g., headache, nausea, and vomiting), but the frequency and severity of adverse effects appear to be lower for cabergoline. Although both drugs are effective in

treating hyperprolactinemia, 12 % patients cannot tolerate bromocriptine [4].

Traditional Chinese medicine uses *Radix bupleuri* extract (RBE) to treat hyperprolactinemia without the toxic side effects associated with conventional anti-hyperprolactinemia agents. In a previous study, we found that RBE decreased prolactin levels in hyperprolactinemic mice [5]. However, the mechanism underlying the anti-hyperprolactinemia effects of RBE require further investigation.

Most conventional anti-hyperprolactinemia agents decrease prolactin secretion through the hypothalamic dopaminergic system, and these effects are mediated via the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway [6]. In this study, we hypothesized that RBE alleviates hyperprolactinemia through modulation of the dopamine D2 receptor. We investigated the effects of RBE on protein levels of dopamine D2 receptor, PKA, and cAMP in the hypothalamus of hyperprolactinemic rats.

EXPERIMENTAL

Preparation of RBE

Samples of *Radix bupleuri* were collected in May 2015 in Bozhou City, Anhui Province, China. Taxonomic identification of the plant was performed by Professor Pei Chen of Changzhi Medical College (Shanxi, China). A voucher specimen (No. RBE 201505034) was deposited in Changzhi Medical College for future reference. One batch of *R. bupleuri* was dried in an oven. An aqueous extract of RBE was prepared by steeping the dried *R. bupleuri* in hot water (60 °C) three times (1 h each time). The extract was dried first in an oven and then freeze-dried. The yield was 62.5 % (1 g powder was obtained from approximately 1.6 g crude sample).

Animals

Female Wistar rats, weighing 200–220 g, were obtained from Shanxi Center for Disease Control and Prevention (Taiyuan, Shanxi). The animals had free access to food and water and were allowed to acclimatize for at least 1 week before experimental procedures. All experiments were approved by the Animal Care and Use Committee of Zhejiang Cancer Hospital (approval ref no. 20120622) and were carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [7].

The rats were treated with metoclopramide (150 mg/kg administered intraperitoneally daily for 10 days), a dopamine inhibitor widely used to generate animal models of hyperprolactinemia [8,9]. A total of 60 rats were divided into six groups (n = 10 per group): rats that did not receive metoclopramide (healthy controls), rats that received metoclopramide only (untreated hyperprolactinemic rats), hyperprolactinemic rats treated with 0.6 mg/kg bromocriptine (positive controls), and hyperprolactinemic rats treated with a high, medium, or low dose of RBE (19.2 g/kg, 9.6 g/kg, or 4.8 g/kg, respectively). The drugs were dissolved in 2 mL water and administered by intragastric gavage; healthy control and untreated hyperprolactinemic rats received 2 mL distilled water. RBE dosages were calculated from the human dosage based on body surface area. The mice were treated for 30 days.

Western blot analysis

To determine hypothalamic dopamine D2 receptor protein levels after the 30-day treatment period, rat hypothalamus tissues were homogenized in RIPA buffer with protease inhibitor (cOmplete EDTA-free protease inhibitor cocktail tablets; Roche Applied Science, Mannheim, Germany) and centrifuged at 3000 × g for 15 min at 4 °C. The supernatant was centrifuged at 12,000 × g for 20 min at 4 °C, and the samples were then diluted in RIPA buffer and loading buffer. After separation by 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis, the proteins were transferred onto polyvinylidene difluoride membranes (Merck). Nonspecific protein-binding sites were blocked by incubating with phosphate-buffered saline containing 0.1 % Tween-20 and 5 % fat-free milk for 1 h at room temperature, followed by incubation with primary antibodies against dopamine receptor D2 (1:1000) and ß-actin (1:5000), which served as a loading control. The membranes were then incubated with lumiGLO reagent (Cell Signaling Technology) and exposed to x-ray film (Kodak, Stamford, CT, USA). Protein bands were quantified by ImageJ (National Institutes of Health, Bethesda, MD, USA). Relative D2 receptor protein levels were determined by normalization to ß-actin.

Enzyme-linked immunosorbent assay

To determine hypothalamic cAMP and PKA protein levels after the 30-day treatment, rat brains were stripped of membranes and blood vessels, homogenized, and then centrifuged for 15 min at 3000 r·min^{-1}. Protein levels of PKA and cAMP in the supernatant were then determined

by enzyme-linked immunosorbent assay (ELISA; Nanjing Jiancheng Biological Technology Co., Ltd, China).

Statistical analysis

Treatment groups were compared by one-way analysis of variance (ANOVA), followed by Student–Newman–Keuls post hoc test for multiple comparisons. Data are expressed as mean ± SEM; $p < 0.05$ was considered statistically significant.

RESULTS

Effects of RBE on hypothalamus dopamine D2 receptor levels in hyperprolactinemic rats

Results of western blot analysis showed that hypothalamus dopamine D2 receptor protein levels were considerably lower in untreated hyperprolactinemic rats than in healthy controls ($p < 0.01$). As expected, this decrease was attenuated by 30-day treatment with 0.6 mg/kg bromocriptine (positive control, $p < 0.01$). Treatment with RBE resulted in a dose-dependent decrease in dopamine D2 receptor levels (4.8 g/kg, $p < 0.05$; 9.6 g/kg, $p < 0.01$; 19.2 g/kg, $p < 0.01$) (Fig. 1).

Effect of RBE on hypothalamus PKA and cAMP levels in hyperprolactinemic rats

Results of ELISA showed that hypothalamus levels of PKA and cAMP were significantly higher in hyperprolactinemic rats compared with healthy controls ($p < 0.01$). This upregulation of PKA and cAMP protein expression was attenuated by treatment with bromocriptine ($p < 0.01$). Treatment with RBE resulted in a dose-dependent decrease in PKA and cAMP levels (4.8 g/kg, $p < 0.05$; 9.6 g/kg, $p < 0.01$; 19.2 g/kg, $p < 0.01$) (Fig. 2).

DISCUSSION

In a previous study, we found that RBE significantly decreased prolactin levels in a mouse model of hyperprolactinemia. In the present study, we investigated the mechanism underlying the anti-hyperprolactinemia effects of RBE and found that RBE significantly increased dopamine D2 receptor protein levels and significantly decreased cAMP and PKA protein levels in the hypothalamus of hyperprolactinemic rats.

Dopamine receptors are members of the G-protein coupled receptor family [10-12] and are classified on the basis of their effect on adenylyl cyclase, which regulates the cAMP-PKA pathway.
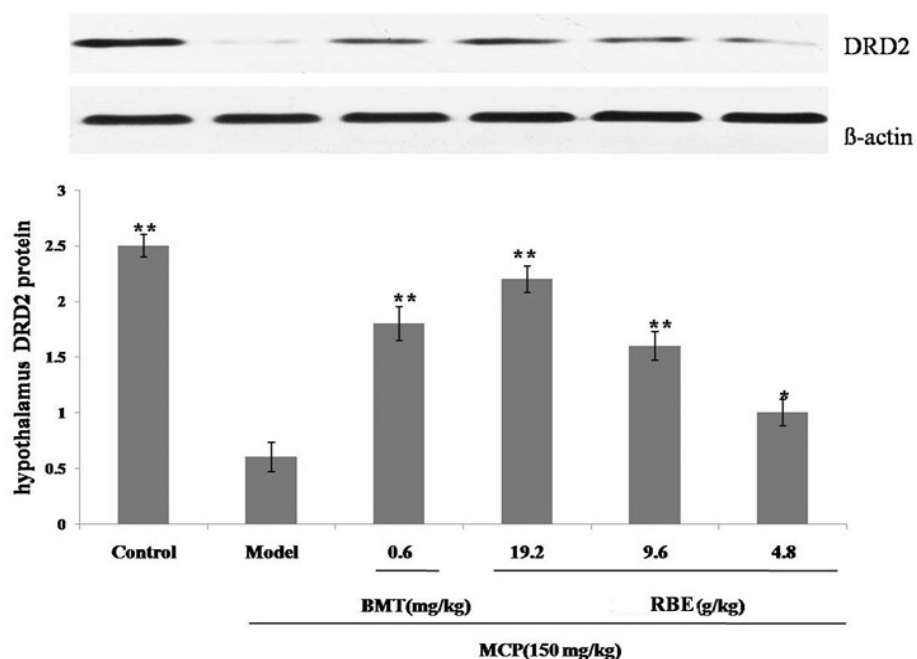
Figure 1: Elevated hypothalamus dopamine D2 receptor (DRD2) levels in metoclopramide-induced hyperprolactinemic rats (Model) were decreased in a dose-dependent manner by *Radix bupleuri* extract (RBE). Results were analyzed by one-way ANOVA and expressed as mean ± SEM (n = 10). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with untreated hyperprolactinemic rats. Control = healthy rats; BMT = bromocriptine-treated hyperprolactinemic rats
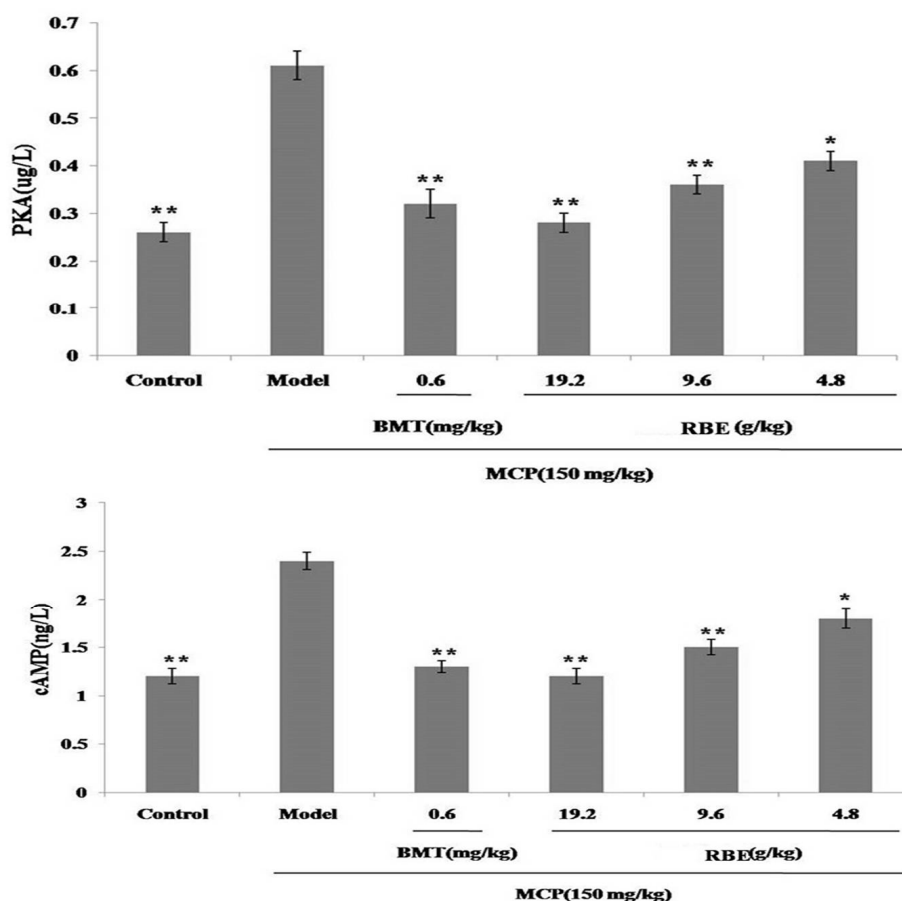
Figure 2: Decreased hypothalamus PKA and cAMP levels in metoclopramide-induced hyperprolactinemic rats (Model) were dose-dependently increased after 30-day treatment with *Radix bupleuri* extract (RBE). Results were analyzed by one-way ANOVA and expressed as mean ± SEM (n = 10). $^*p < 0.05$ and $^{**}p < 0.01$ compared with untreated hyperprolactinemic rats. Control = healthy rats; BMT = bromocriptine-treated hyperprolactinemic rats

D1-like dopamine receptors (D1 and D5) couple primarily to Gαs and increase intracellular levels of the second messenger cAMP and upregulate the activity of PKA. By contrast, D2-like dopamine receptors (D2, D3, and D4) couple to Gαi/o and decrease levels of cAMP, thereby suppressing PKA activity [13–15].

In the adenohypophysis, which secretes prolactin, the predominant dopamine receptor is the D2 receptor [16-18]. In a pituitary cell line transfected with the gene encoding the dopamine D2 receptor, treatment with dopamine decreased intracellular cAMP levels and inhibited prolactin secretion [19,20]. Similarly, most conventional anti-hyperprolactinemia agents are D2 receptor agonists, which decrease prolactin secretion through cAMP/PKA signaling. In this study, RBE increased dopamine D2 receptor levels and decreased cAMP and PKA levels in the hypothalamus of hyperprolactinemic rats significantly. These results suggest that the anti-hyperprolactinemic activity of RBE is mediated by dopamine D2 receptor and cAMP/PKA signaling.

CONCLUSION

Radix bupleuri extract decreases prolactin secretion in hyperprolactinemic rats via dopamine D2 receptor and cAMP/PKA signaling. This mechanism is similar to that of conventional treatments for hyperprolactinemia, without the toxic side effects. This traditional Chinese medicine may, therefore, be useful for the treatment of patients with hyperprolactinemia.

DECLARATIONS

Acknowledgement

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. Lappohn RE, van de Wiel HB, Brownell J. The effect of two dopaminergic drugs on menstrual function and psychological state in hyperprolactinemia. Fertil Steril 1992; 58: 321–327.
2. Biller BM, Luciano A, Crosignani PG. Guidelines for the diagnosis and treatment of hyperprolactinemia. J Reprod Med 1999; 44: 1075-1084.
3. Greer ME, Moraczewski T, Rakoff JS. Prevalence of hyperprolactinemia in anovulatory women. Obstet Gynecol 1980; 56: 65-69.
4. Webster J, Piscitell G, Polli A. A Comparison of Cabergoline and Bromocriptine in the Treatment of Hyperprolactinemic Amenorrhea. N Engl J Med 1994; 331: 904-909.
5. Zhang ZJ, Tan QR, Zhen XC, Tong Y. The potential benefits of herbal medicines for schizophrenia: from empirical observations to clinical trials (chapter 16). In: Hertzman M, Adler L, Eds, Clinical Trials in Psychopharmacology. UK: Wiley-Blackwell 2010; pp 311–335.
6. Wang X, Chen YG, Ma L, Wu JH. Effect of Chinese Medical Herbs-Huiru Yizeng Yihao on Hyperprolactinemia and Hyperplasia of Mammary Gland in Mice. Afr J Tradit Complement Altern Med 2013; 10: 24-35.
7. Fitzgerald P, Dinan TG. Prolactin and dopamine: What is the connection? J Psychopharmacol 2008; 22(2S): 12–19.
8. European Commission [homepage on the internet]. Directive 2010/63/EU on the protection of animals used for scientific purposes [cited 2013 Jan 16]. Available from: http://ec.europa.eu/environment/chemicals/lab Animals/legislation_en.htm.
9. Laszczyńska M, Suczanowska-Gabowska S, Piasecka M, Skowron J, Debińska-Szymańska T. Germ cells with nuclear DNA fragmentation related to apoptotic cells in rat testis in experimental hyperprolactinemia induced by metoclopramide. Folia Histochem Cytobiol 2002; 40: 163-164.
10. Gingrich JA, Caron MG. Recent advances in the molecular biology of dopamine receptors. Annu. Rev. Neurosci 1993; 16: 299-321.
11. Li HZ, Guo J, Gao J. Role of dopamine D2 receptors in ischemia/reperfusion induced apoptosis of cultured neonatal rat cardiomyocytes. J. Biomed. Sci. 2011; 16: 18-20.
12. Li HZ, Han LP, Jiang CM. Effect of dopamine receptor 1 on apoptosis of cultured neonatal rat cardiomyocytes in simulated ischaemia/reperfusion. Basic. Clin. Pharmacol. Toxicol. 2008; 102: 329-336.
13. Missale C. Dopamine receptors: from structure to function. Physiol. Rev. 1998; 78: 189-225.
14. Greengard P. The neurobiology of slow synaptic transmission. Science 2001; 294: 1024–1030.
15. Beaulieu JM, Gainetdinov RR, Caron MG. The Akt–GSK-3 signaling cascade in the actions of dopamine. Trends. Pharmacol. Sci. 2007; 28: 166-172.
16. Madras BK. History of the discovery of the antipsychotic dopamine d2 receptor: a basis for the dopamine hypothesis of schizophrenia. J. Hist. Neurosci. 2013; 22: 62–78.
17. Narkar V, Kunduzova O, Hussain T, Cambon C. Dopamine D2-like receptor agonist bromocriptine protects against ischemia/reperfusion injury in rat kidney. Kidney Int. 2004; 66: 633–634.
18. Cronin MJ. The role and direct measurement of the dopamine receptor(s) in the anterior pituitary. In: Muller EE, MacLeod RM, Eds. Neuroendocrine perspectives 1982; 1: 169-210.
19. Nilsson C, Eriksson E. Partial dopamine D2 receptor agonists antagonize prolactin regulating D2 receptors in a transfected clonal cell line (GH4ZR7). Eur J pharmacol 1992; 218: 205–211.
20. Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, Brue T. Guidelines of the Pituitary Society for the diagnosis and management of prolactinomas. Clin Endocrinol 2006; 65: 265–273.