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Inhibitory effects of bromocriptine on corticosterone secretion in male rats

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Abstract

Bromocriptine, a dopamine D2 receptor agonist, is widely used for treating prolactinoma, Parkinson's disease and galactorrhea. However, the influence of bromocriptine on the endocrine system, especially adrenal function, is not clear. The present study was aimed to investigate the effects of bromocriptine on corticosterone production in rats. Male rats were treated or not treated by bromocriptine (5 mg/kg, s.c.) twice per day for 2 days before decapitation. The adrenal zona fasciculata–reticularis cells were prepared and incubated with adrenocorticotropic hormone (ACTH), forskolin (an adenylyl cyclase activator), 8-bromo-adenosine 3′5′ cyclic monophosphate (8-Br-cAMP, a membrane-permeable analogue of cAMP), and steroidogenic precursors including 25-OH-cholesterol and pregnenolone. The concentrations of prolactin, corticosterone and pregnenolone in the plasma and/or medium were measured by radioimmunoassay (RIA). The protein expression of cytochrome P450 side-chain cleavage (P450scc) enzyme and steroidogenic acute regulatory protein (StAR) was analyzed by Western blotting. Administration of bromocriptine in vivo resulted in a decrease in the levels of plasma prolactin and corticosterone. Basal—and ACTH—as well as forskolin-stimulated corticosterone secretion by zona fasciculata–reticularis cells was also lower in bromocriptine-treated rats than in control animals. The decreased production of corticosterone in zona fasciculata–reticularis cells could be reversed by administration of 8-Br-cAMP. The corticosterone and pregnenolone release induced by 25-OH-cholesterol in zona fasciculata–reticularis cells was reduced by administration of bromocriptine. The protein expression of both StAR protein and P450scc in zona fasciculata–reticularis cells was inhibited in the bromocriptine-treated group. Administration of bromocriptine in vitro reduced the release of corticosterone stimulated by ACTH and forskolin in rat zona fasciculata–reticularis cells. These results suggested that bromocriptine caused adrenal dysfunction through inhibition of ACTH action and of the activity of adenylyl cyclase, and impaired the early steps of corticosterone biosynthesis.

Keywords: Bromocriptine; Corticosterone; Zona fasciculata–reticularis cell; Adenylyl cyclase; Cytochrome P450scc; StAR protein

1. Introduction

Bromocriptine, an ergot alkaloid derivative, structurally related to dopamine and suggested to be a dopamine D2 receptor agonist (Barbieri and Ryan, 1983). Bromocriptine is widely used clinically for the treatment of patients with microprolactinoma and macroprolactinoma. With this drug treatment, serum prolactin concentration usually dropped to the normal range (Barbieri and Ryan, 1983). Bromocriptine directly binds to pituitary dopamine D2 receptors and inhibits prolactin secretion through the inhibition of adenylyl cyclase activity (Barbieri and Ryan, 1983; Kebabian et al., 1986; Vanllar and Meldolesi, 1989). Bromocriptine is also a common drug for the therapy of Parkinson's disease and most forms of galactorrhea (Lancranjan, 1981; Ogo et al., 1993; Ahlskog, 1994; Ogawa, 1998). Previous reports showed that bromocriptine exerts pharmacological effects on the nervous system. Bromocriptine decreases the spontaneous firing rate of neurons in cortex and the pars compacta of the substantia nigra (Bioulac et al., 1978; Scarnati et al., 1980). Tuberoinfundibular dopaminergic neuron activity is inhibited by bromocriptine treatment (Demarest et al., 1985). Bromocriptine reduces adenylyl cyclase activity in olfactory sensory neurons (Mania-Faenell et al., 1993). The side-effects of bromocriptine on cardiovascular system have also been investigated. Treatment with bromocriptine decreases blood pressure and heart rate in both experimental animals and in humans (Hof and Hof,

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1984; Ageel et al., 1987; Roquebert et al., 1991; Schobel et al., 1995). Furthermore, cases of constrictive pericarditis induced by bromocriptine have been reported (Champagne et al., 1999).

In the endocrine system, bromocriptine dramatically reduces the plasma prolactin concentration and induces hypoprolactinemia (Dissaneevate and Warne, 1998). Previous reports showed that bromocriptine-induced hypoprolactinemia alters not only corpus luteum formation and ovary function in female rats (Kauppila et al., 1988) but also luteinizing hormone (LH) and prolactin receptor expression in male rats (Pakarinen et al., 1994). Meanwhile, results of numerous studies indicated that bromocriptine affects adrenal function in many animal species. In prepubertal rats, injection of the highest dose of bromocriptine results in increased adrenal weight (Luthy et al., 1984). Treatment with bromocriptine for 6 days significantly reduced both prolactin and cortisol concentrations in the plasma of castrated pigs (Klemcke et al., 1990). These findings implied that bromocriptine not only affects nervous and cardiovascular systems but also influences the endocrine systems, such as adrenal function. Therefore, the possible effects and mechanism(s) of action of bromocriptine on adrenal glands should be investigated.

The present study was designed to clarify the side-effects and the mechanism(s) of action of bromocriptine on corticosterone release by rat zona fasciculata–reticularis cells. We found that bromocriptine given in vivo decreased corticosterone release through mechanisms involving an attenuation of adrenocorticotropic hormone (ACTH) action, and of adenylyl cyclase activity. Furthermore, both the activity and protein expression of steroidogenic acute regulatory (StAR) protein and cytochrome P450 side-chain cleavage enzyme (P450scc), the key enzymes of the steroidogenic biosynthetic pathway, were attenuated by bromocriptine in rat zona fasciculata–reticularis cells.

2. Materials and methods

2.1. Animals

Male rats of the Sprague–Dawley strain weighing 300–350 g were housed in a temperature-controlled room (22 ± 1 °C) with 14 h of artificial illumination daily (0600–2000 h). Food and water were given ad libitum. All animal experimentation was conducted in conformance with the policy statement of the Committee of National Yang-Ming University.

2.2. Preparation of zona fasciculata–reticularis cells

An adrenocortex preparation enriched with zona fasciculata–reticularis cells for culture was made following a method described elsewhere by Purdy et al. (1991) with minor modification (Lo et al., 1998a,b; Chang et al., 1999, 2002). After the animals were decapitated, the adrenal glands were rapidly excised and stored in ice-cold 0.9% NaCl solution. The encapsulated glands were separated into outer zone (mainly zona glumerulosa) and inner zone (mainly zona fasciculata and zona reticularis) fractions with forceps. The fraction of inner zone from six to eight adrenals was incubated with collagenase (2 mg/ml) at 37 °C in a vibrating water bath, 100–110 strokes/min, for 60 min. The collagenase was dissolved in 2–4 ml of Krebs–Ringer bicarbonate buffer (3.6 mmol K^+/l, 11.1 mmol glucose/l) with 0.2% bovine serum albumin (KRBGA), pH 7.4. The zona fasciculata–reticularis cells were dispersed by repeated pipetting and filtered through a nylon mesh. After centrifugation at 200 × *g* for 10 min, the cells were washed in KRBGA medium and centrifuged again. Erythrocytes were separated by hypotonic shock with 9 ml deionized water for a few seconds. The zona fasciculata–reticularis cells were then mixed with 1 ml of 10 × Hank's balanced salt solution (HBSS, pH 7.4). After centrifugation at 200 × *g* for 10 min, the supernatant was discarded and the pellets were resuspended in 3 ml of KRBGA medium. An aliquot (20 μl) was used to count the cells in a hemocytometer after staining with 0.05% nigrasin stain. The viability of isolated zona fasciculata–reticularis cells was 70–75%. Cells in the culture medium were further diluted to a concentration of 5×10^4 cells/ml and the suspension was divided among the test tubes.

2.3. Experiment: effects of bromocriptine in vivo on corticosterone release by zona fasciculata–reticularis cells

Male rats were injected subcutaneously (s.c.) with vehicle (35% alcohol) or 5 mg/ml/kg bromocriptine twice per day for 2 days. Two hours after the last injection of vehicle or bromocriptine, the rats were decapitated. Trunk blood was collected and the plasma was separated by centrifugation at 1000 × *g* for 30 min at 4 °C. The concentrations of prolactin and corticosterone in plasma samples were measured by radioimmunoassay (RIA).

To measure the effects of bromocriptine in vivo on cyclic AMP-related corticosterone release, the zona fasciculata–reticularis cells (5×10^4 cells/ml) were preincubated at 37 °C under 95% O_2–5% CO_2 for 60 min prior to incubation with 0.5 ml medium containing ACTH-(1–24) (10^{-10} and 10^{-9} M), forskolin (adenylyl cyclase activator, 10^{-6} and 10^{-5} M) or 8-Br-cAMP (a membrane-permeable analog of cyclic AMP, 10^{-5} and 10^{-4} M) for 60 min. At the end of the incubation, the incubation tubes were centrifuged at 100 × *g* for 10 min at 4 °C. The supernatant was collected for measurement of corticosterone by RIA.

For studying the influence of bromocriptine on the early step of enzyme activity of steroidogenesis, rat zona fasciculata–reticularis cells were incubated with 0.5 ml KRBGA medium containing precursors of steroidogenesis such as 25-OH-cholesterol (10^{-5} M) or pregnenolone (10^{-5} M) for 1 h. The media were collected, and the

concentrations of corticosterone and pregnenolone were measured by RIA.

2.4. Experiment: effects of bromocriptine in vitro on corticosterone production by rat zona fasciculata–reticularis cells

Normal rats were decapitated. The zona fasciculata–reticularis cell suspensions (5×10^4 cell/ml) were prepared, preincubated with 1 ml KRBGA medium for 60 min, and then incubated with 0.5 ml medium containing bromocriptine (0, 10^{-7}–10^{-5} M) in the presence or absence of ACTH (10^{-9} M), forskolin (10^{-5} M) or 8-Br-cAMP (10^{-4} M) for 60 min. The media were collected and the concentrations of corticosterone were measured by RIA.

2.5. Corticosterone RIA

The concentrations of corticosterone in both plasma and medium were determined by RIA as described elsewhere (Chen et al., 1997; Lo et al., 1998a,b; Chang et al., 2002). With anti-corticosterone No. PSW# 4-9, the sensitivity of corticosterone was 5 pg/assay tube. The intra- and interassay coefficients of variation were 3.3% ($n=5$) and 9.5% ($n=4$), respectively.

2.6. Pregnenolone RIA

The concentrations of pregnenolone were measured by RIA. The cross-reactivities of the anti-pregnenolone antibody were 67% with pregnen-36-OL-20-ONE sulphate, 19% with progesterone, and <3% with 17α-hydroxypregnenolone, cholesterol, 17α-OH-progesterone, 20α-diOH-progesterone, cortisol, deoxycorticosterone, corticosterone, aldosterone, androstenedione, testosterone, estradiol, estrone or estriol. For the RIA system, a known amount of unlabeled pregnenolone or an aliquot of rat zona fasciculata–reticularis cell medium, adjusted to a total volume of 0.3 ml with a buffer solution [0.1% gelatin-phosphate-buffered saline (PBS), pH 7.5], was incubated with 0.1 ml pregnenolone antiserum diluted with 0.1% gelatin-PBS plus 0.1 ml [^{3}H]pregnenolone at 4 °C for 24 h. Duplicate standard curves for pregnenolone were incubated in each assay. An adequate amount (0.1 ml) of dextran-coated charcoal (0.5%) was added and further incubated in an ice bath for 15 min. After incubation, the assay tubes were centrifuged at $1500 \times g$ for 40 min. The supernatant was mixed with 3 ml liquid scintillation fluid (Ready Safe) before the radioactivity was counted in an automatic beta counter (Wallac 1409, Pharmacia, Turku, Finland). The sensitivity of the pregnenolone RIA was 16 pg/assay tube. The inhibition curves produced by zona fasciculata–reticularis cell medium samples were parallel to those produced by pregnenolone. The intra- and interassay coefficients of variation were 2.5% ($n=4$) and 3.9% ($n=5$), respectively.

2.7. Western blotting

The zona fasciculata–reticularis cells were collected from vehicle and bromocriptine groups and washed three times with saline. The cells were lysed in lysis buffer consisting of 1.5% Na-lauroylsarcosine, 2.5×10^{-3} mol/l Tris–base, 1×10^{-3} mol/l EDTA, 0.68% phenylmethylsulfonyl fluoride and 2% proteinase inhibitors, pH 8.0, and disrupted by sonication in an ice-bath. The cell lysate was centrifuged for 12 min at $10{,}000 \times g$ and the supernatant (cell extract) was collected. Total protein was determined by Bradford protein assay (Zor and Selinger, 1996). The protein was denatured by boiling for 10 min in sodium dodecyl sulphate (SDS) buffer (0.125 mol/l, 12% sucrose, 0.15 mol/l dithiothreitol). Western blotting and gel electrophoresis were performed as previously described (Kau et al., 1999) and the proteins were separated by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to a polyvinylidene difluoride membrane. The membranes were washed with TBS-T buffer (0.8% NaCl, 0.02 mol/l Tris–base and 0.3% Tween-20, pH 7.6) for 5 min and then blocked for 60 min in blocking buffer (TBS-T buffer containing 5% nonfat dry milk) at room temperature. Then, the membranes were immunoblotted with primary antibodies (rabbit polyclonal antibodies to P450scc 1:1000, mouse monoclonal antibodies to StAR 1:1000 and β-actin 1:8000). Primary antibodies were

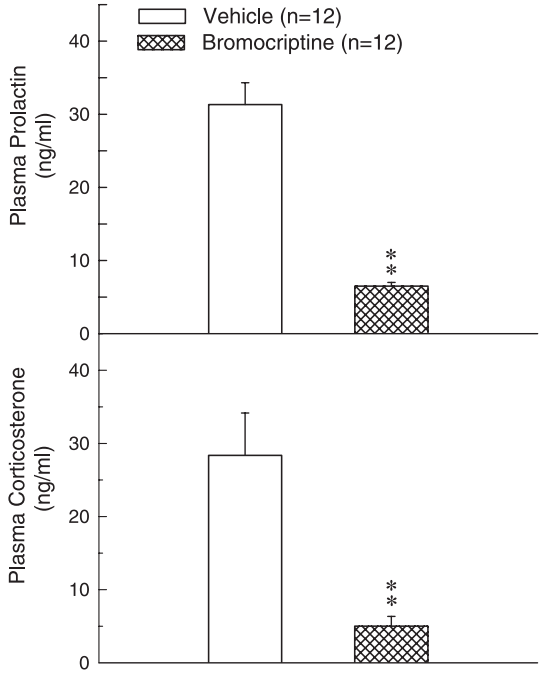

Fig. 1. Effects of bromocriptine given in vivo on plasma concentration of prolactin and corticosterone in male rats. Rats were injected with vehicle (40% alcohol, blank column, $n=12$) or bromocriptine (5 mg/kg) twice daily (hatched column, $n=12$) before decapitation. Each column represents the mean ± S.E.M. $^{**}P<0.01$ compared to vehicle group.

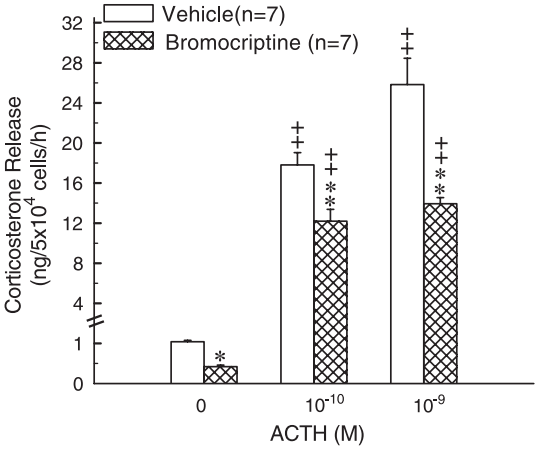

Fig. 2. Effects of ACTH on corticosterone release in zona fasciculata–reticularis cells isolated from vehicle- and bromocriptine-treated rats. Each column represents the mean ± S.E.M. *$P<0.05$ and **$P<0.01$ compared to vehicle group. $^{++}P<0.01$ versus corresponding basal release (ACTH = 0 M).

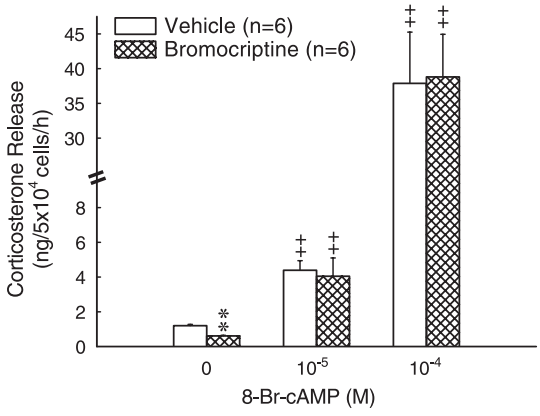

Fig. 4. Effects of 8-Br-cAMP on corticosterone release in zona fasciculata–reticularis cells isolated from vehicle- and bromocriptine-treated rats. Each column represents the mean ± S.E.M. **$P<0.01$ compared to vehicle group; $^{++}P<0.01$ versus corresponding basal release (8-Br-cAMP = 0 M).

detected using anti-rabbit and anti-mouse horseradish peroxidase-conjugated secondary antibodies in 5% nonfat skim milk in TBS-T buffer. The membranes were washed with TBS-T buffer and then the bands of StAR or P450scc were visualized by enhanced chemiluminescence (ECL) detection.

2.8. Materials

ACTH-(1–24), bovine serum albumin, bromocriptine, collagenase, forskolin, 8-Br-cAMP, HBSS, 25-OH-cholesterol, β-actin antibodies and pregnenolone were purchased from Sigma (St. Louis, MO, USA). The anti-pregnenolone antiserum was purchased from Biogenesis (Sandown, NH, USA). The horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) and goat anti-mouse IgG were from ICN Pharmaceuticals (Aurora, OH, USA). The ECL detection kits and [^{3}H]pregnenolone were obtained from Amersham International (Buckinghamshire, UK). The liquid scintillation fluid (Ready Safe) was from Beckman (Fullerton, CA, USA). The polyvinylidene difluoride membranes were purchased from NEN Life Science Products (Boston, MA, USA). ECL™ Western blotting detection reagents were from Amersham Pharmacia Biotech. (Bucks, UK). The anti-P450scc antibody and anti-StAR antibody were kindly provided by Dr. B.C. Chung (Hu et al., 1991) and Dr. D.M. Stocco (Lin et al., 1998), respectively.

2.9. Statistical analysis

All values are given as the means ± S.E.M. In some cases, the treatment means were tested for homogeneity by

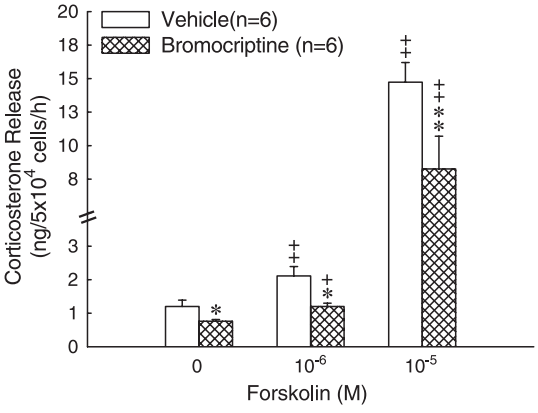

Fig. 3. Effects of forskolin on corticosterone release in zona fasciculata–reticularis cells isolated from vehicle- and bromocriptine-treated rats. Each column represents the mean ± S.E.M. *$P<0.05$ and **$P<0.01$ compared to vehicle group. $^{+}P<0.05$ and $^{++}P<0.01$ versus corresponding basal release (forskolin = 0 M).

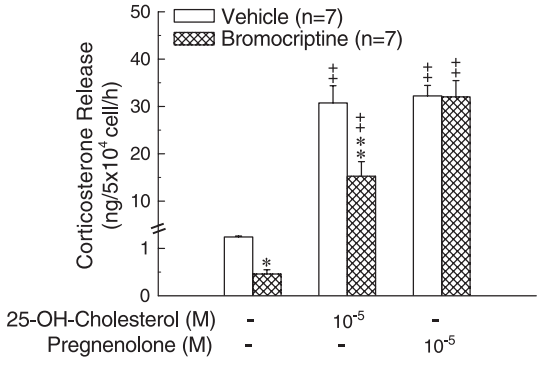

Fig. 5. Effects of 25-OH-cholesterol or pregnenolone on corticosterone release in zona fasciculata–reticularis cells isolated from vehicle- and bromocriptine-treated rats. Each column represents the mean ± S.E.M. *$P<0.05$ and **$P<0.01$ compared to vehicle group, respectively; $^{++}P<0.01$ versus corresponding basal release.

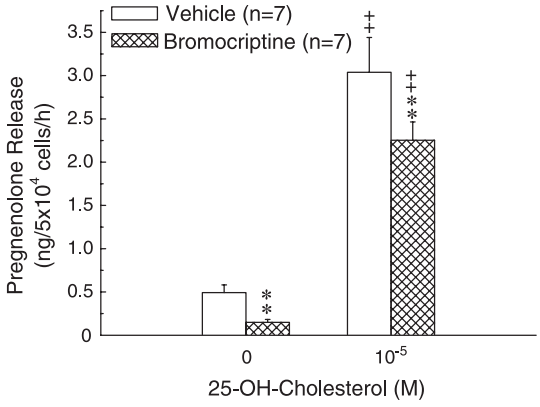

Fig. 6. Effect of 25-OH-cholesterol on pregnenolone secretion by zona fasciculata–reticularis cells isolated from vehicle- and bromocriptine-treated rats. Each column represents the mean ± S.E.M. $^{**}P<0.01$ compared to vehicle group; $^{++}P<0.01$ versus corresponding basal release.

one-way analysis of variance (ANOVA) and the difference between specific means was tested for significance by Duncan's multiple-range test (Steel and Torrie, 1960). In other cases, Student's *t*-test was employed. A difference between two means was considered statistically significant when $P<0.05$.

3. Results

3.1. Effects of bromocriptine in vivo on the plasma levels of prolactin and corticosterone

After administration of bromocriptine (5 mg/ml/kg) twice daily for 2 days, plasma prolactin concentration was significantly decreased (6.50 ± 0.50 ng/ml, $n=12$, $P<0.01$) compared to the vehicle value (31.33 ± 6.50 ng/ml, $n=12$) (Fig. 1). Accompanying the lower level of plasma prolactin concentration, a decreased level of plasma corticosterone concentration was observed in the bromocriptine group (5.01 ± 1.33 ng/ml versus vehicle group 28.36 ± 5.80 ng/ml, $n=12$, $P<0.01$).

3.2. Effects of bromocriptine in vivo on cyclic AMP-related corticosterone release

Incubation with ACTH (10^{-9} or 10^{-10} M) or forskolin (10^{-6} or 10^{-5} M) significantly ($P<0.01$) increased corticosterone release by zona fasciculata–reticularis cells in both vehicle- and bromocriptine-treated groups (Figs. 2 and 3). ACTH increased corticosterone secretion by 18.22 ± 1.36 to 27.04 ± 2.90, 12.97 ± 1.30 to 14.88 ± 0.68 ng/5 × 10^4 cells/h in zona fasciculata–reticularis cells from the

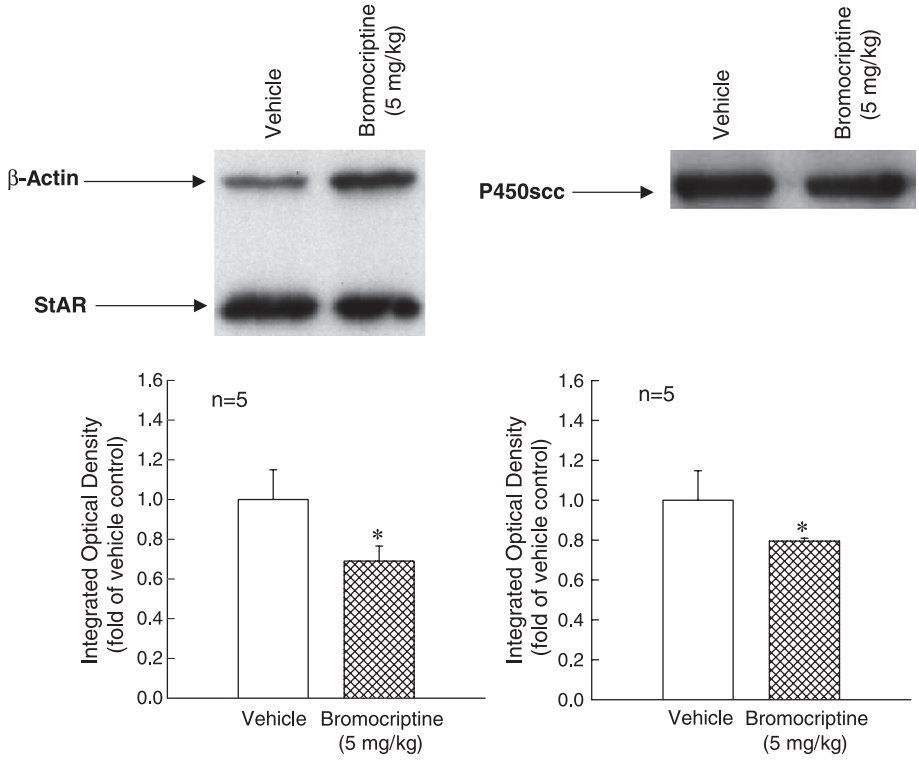

Fig. 7. Protein expression of steroidogenic acute regulatory (StAR) protein and cytochrome P450 side chain cleavage enzyme (P450scc). Vehicle- and bromocriptine-treated rats were decapitated and adrenal zona fasciculata–reticularis cells were isolated. Zona fasciculata–reticularis cells were homogenized and total protein concentration was determined with the Bradford assay. Sample proteins were separated by SDS-PAGE and immunoblotted with anti-StAR, anti-P450scc and anti-β-actin antibodies. Each column represents the mean ± S.E.M. $^{*}P<0.05$ compared to vehicle group.

vehicle- and bromocriptine-treated rats, respectively. Forskolin stimulated corticosterone release by 0.91 ± 0.19 to 13.53 ± 1.47, 0.44 ± 0.10 to 7.76 ± 2.44 ng/5 × 10^4 cells/h from the vehicle- and bromocriptine-treated rats, respectively. The net increases of corticosterone secretion in response to ACTH or forskolin were lower in bromocriptine-treated than in vehicle groups. Application of 8-Br-cAMP (10^{-5} or 10^{-4} M) significantly enhanced corticosterone release in both vehicle and bromocriptine group ($P<0.01$, Fig. 4). However, there were no significant differences in 8-Br-cAMP-increased corticosterone secretion in zona fasciculata–reticularis cells between vehicle- and bromocriptine-treated groups.

3.3. Effects of bromocriptine in vivo on the enzyme activities of P450scc and 3β-hydroxysteroid dehydrogenase, 21-hydroxylase and 11β-hydroxylase

Incubation of rat zona fasciculata–reticularis cells with 25-OH-cholesterol (10^{-5} M) or pregnenolone (10^{-5} M) for 1 h markedly increased corticosterone release not only in the vehicle group but also in the bromocriptine-treated group ($P<0.01$, Fig. 5). Compared with that in the vehicle group, the net increase in corticosterone secretion stimulated by 25-OH-cholesterol was lower in the bromocriptine-treated group (14.85 ± 3.09 ng/5 × 10^4 cells/h, $n=7$, $P<0.01$) than in the vehicle group (29.50 ± 3.46 ng/5 × 10^4 cells/h, $n=7$). There was no difference in corticosterone release in response to pregnenolone between vehicle- and bromocriptine-treated groups (Fig. 5). The results implied that the enzyme activities of 3β-hydroxysteroid dehydrogenase, 21-hydroxylase and 11β-hydroxylase were unchanged by bromocriptine treatment.

Since the corticosterone release stimulated by 25-OH-cholesterol was lower in bromocriptine-treated rats, the concentration of pregnenolone in the media was measured. Application of 25-OH-cholesterol significantly increased pregnenolone secretion in the vehicle- and the bromocriptine-treated groups. The net increased level of pregnenolone stimulated by 25-OH-cholesterol showed that the bromocriptine-treated group (3.36 ± 0.43 ng/5 × 10^4 cells/h, $n=7$, $P<0.01$) had a decreased response to 25-OH-cholesterol compared with that of the vehicle group (4.59 ± 0.81 ng/5 × 10^4 cells/h, $n=7$, $P<0.01$, Fig. 6).

3.4. Effects of bromocriptine in vivo on the protein expression of StAR protein and P450scc

The protein expression of StAR and P450scc was analyzed by Western blotting. After administration of bromocriptine in vivo, the level of StAR protein expression in zona fasciculata–reticularis cells was 65% of the corresponding control ($P<0.05$, Fig. 7). Suppression similar to that of StAR expression was observed for P450scc protein expression. Inhibition up to 80% of the control was observed after treatment with bromocriptine in vivo ($P<0.05$, Fig. 7).

3.5. Effects of bromocriptine in vitro on corticosterone release by zona fasciculata–reticularis cells

Administration of bromocriptine in vitro (10^{-7}–10^{-5} M) did not alter the basal release of corticosterone by zona fasciculata–reticularis cells (1.24 ± 0.18 to 1.44 ± 0.30 ng/5 × 10^4 cells/h versus basal release 1.23 ± 0.15 ng/5 × 10^4 cells/h, $n=6$) (Fig. 8).

Both ACTH (10^{-9} M) and forskolin (10^{-5} M) caused a significant rise in corticosterone production by zona fasciculata–reticularis cells (Fig. 8, two center panels). Administration of bromocriptine in vitro (10^{-7}–10^{-5} M) dose dependently attenuated the corticosterone release stimulated by ACTH and by forskolin ($P<0.05$ and $P<0.01$). Incubation of zona fasciculata–reticularis cells with 8-Br-cAMP (10^{-4} M) for 1 h increased the release of corticosterone in vitro ($P<0.01$) (Fig. 8, bottom). However, bromocriptine (10^{-7}–10^{-5} M) did not alter the corticosterone release in response to 8-Br-cAMP.

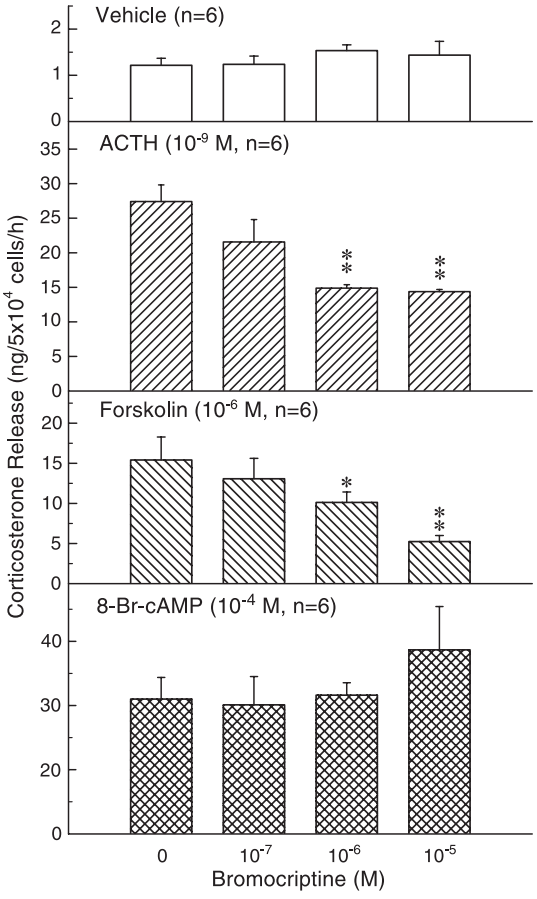

Fig. 8. Effects of bromocriptine in vitro on the basal, ACTH (10^{-9} M)-, forskolin (10^{-5} M)- and 8-Br-cAMP (10^{-4} M)-stimulated corticosterone release in rat zona fasciculata–reticularis cells. Each column represents the mean ± S.E.M. *$P<0.05$ and **$P<0.01$ compared to control group (bromocriptine = 0 M).

4. Discussion

Bromocriptine, an agonist of dopamine, acts at the anterior pituitary to exert an inhibitory effect on prolactin secretion. Bromocriptine has been successfully introduced as therapy for the pituitary hypersecretion syndromes of hyperprolactinemia (Lancranjan, 1981; Vance et al., 1984; Robert and Hardy, 1986; Maor and Berezin, 1997). Meanwhile, outcomes of numerous clinical studies have implied that bromocriptine exerted pharmacological effects on the endocrine system, especially the adrenal glands (Luthy et al., 1984; Klemcke et al., 1990; Ahlskog, 1994). According to our results, the decreased level of plasma prolactin in response to bromocriptine reflects a successfully induced hypoprolactinemia in male rats. Together with the decreased plasma prolactin concentration, a lower level of plasma corticosterone was observed. These findings indicated that bromocriptine resulted in a deficit of adrenal cortical function. To study the possible mechanism(s), isolated adrenal zona fasciculata–reticularis cells were used to explore the in vivo effects of bromocriptine.

In the present study, the corticosterone release in response to ACTH was always lower in bromocriptine-treated animals. It is well known that ACTH regulates glucocorticoid secretion through cyclic AMP pathway (Shima et al., 1979; Moriwari et al., 1982). The findings implied that the inhibitory effects of bromocriptine on corticosterone secretion might be mediated through the cyclic AMP pathway. In order to examine the cyclic AMP pathway mediation of the effect of bromocriptine on adrenal zona fasciculata–reticularis cells, an adenylyl cyclase activator, forskolin, and a membrane permeable cyclic AMP analog, 8-Br-cAMP, were used. After administration of forskolin, the corticosterone release by zona fasciculata–reticularis cells in the bromocriptine-treated group was lower than that in vehicle-treated animals. Decreased adenylyl cyclase activity in zona fasciculata–reticularis cells was observed on application of bromocriptine in vivo. The data suggested that a lower level of corticosterone secretion caused by bromocriptine was associated with a deficiency of adenylyl cyclase activity. However, the corticosterone release stimulated by 8-Br-cAMP was unchanged in zona fasciculata–reticularis cells from vehicle- and bromocriptine-treated rats. The results suggested that the post cyclic AMP pathway seemed not to be disturbed by bromocriptine. Our results suggested that the lower level of plasma corticosterone and that of corticosterone secretion caused by bromocriptine were partially due to an attenuated response of zona fasciculata–reticularis cells to ACTH and an inhibited adenylyl cyclase activity of zona fasciculata–reticularis cells.

It had been indicated that bromocriptine suppresses the plasma ACTH concentration in patients with Cushing disease and Nelson's syndrome (Kapcala and Jackson, 1982; Ogo et al., 1993; Mercado-Asis et al., 1997). There are reports that hyperprolactinemia activates the hypothalamic pituitary adrenal axis. An elevated concentration of plasma corticosterone was observed in hyperprolactinemic rats (Kooy et al., 1990; Calogero et al., 1993). Prolactin receptors have been detected in all three zones of the adrenal cortex (Glasow et al., 1996). Recently, we found that prolactin enhanced corticosterone release by acting directly on rat zona fasciculata–reticularis (Chang et al., 1999). Bromocriptine, a dopamine receptor agonist, markedly decreases the plasma prolactin concentration (Lancranjan, 1981; Dissaneevate and Warne, 1998). Since bromocriptine has been shown to reduce serum ACTH in a variety of species, the reduction in serum corticosterone may be secondary to a reduction in ACTH. However, we have monitored the plasma levels of ACTH and found that bromocriptine given in vivo did not alter the plasma ACTH concentration as compared with that in the vehicle-treated group (data not shown). After application of bromocriptine in vivo, the plasma level of corticosterone was decreased by approximately 83% as compared to the control. About a 48% decrease in basal corticosterone release by zona fasciculata–reticularis cells was observed after bromocriptine treatment. Plasma corticosterone did not parallel the in vitro corticosterone release by zona fasciculata–reticularis cells after bromocriptine treatment. Furthermore, the in vitro data showed that bromocriptine at the highest dose (e.g., 10^{-5} M) resulted in a 49% decrease in ACTH-stimulated corticosterone release. We found that the plasma prolactin concentration was suppressed as compared with that in the vehicle-treated group rather than plasma ACTH. Therefore, we suggest that the lower plasma concentration of corticosterone induced by bromocriptine might be partially mediated through an inhibitory effect of bromocriptine on prolactin secretion rather than an effect of bromocriptine on ACTH release from the anterior pituitary.

In rat adrenal glands, the biosynthesis of corticosterone involves the activation of StAR, P450scc, 3β-hydroxysteroid dehydrogenase, 21-hydroxylase and 11β-hydroxylase (Lieberman et al., 1984; Kallen et al., 1998). During steroidogenesis, the conversion of cholesterol to pregnenolone is the early rate-limiting step. P450scc, locates in the matrix of the inner mitochondrial membrane, catalyzes the cleavage of the side chain of cholesterol to form pregnenolone (Yago and Ichii, 1969). Transfer of cholesterol from outer to inner to mitochondrial membrane is considered to be the rate-limiting step (Crivello and Jefcoate, 1980; Privalle et al., 1983; Miller, 1995). The protein responsible for the rate-limiting step is identified as a 30-kDa protein named StAR protein (Clark et al., 1994; Stocco, 1999). Therefore, both StAR and P450scc play an important role in the regulation of steroidogenesis. In the present study, the production of both corticosterone and pregnenolone by zona fasciculata–reticularis cells in response to 25-OH-cholesterol was lower in bromocriptine- than in vehicle-treated groups. Based on Western blotting, attenuated protein expression of StAR and P450scc was observed in the bromocriptine-treated group. These data indicated that the

protein expressions of StAR and P450scc as well as P450scc activity were impaired in zona fasciculata–reticularis cells of bromocriptine-treated animals. Because the production of corticosterone in response to pregnenolone was not altered by bromocriptine treatment, we suggest that the activity of 3β-hydroxysteroid dehydrogenase, 21-hydroxylase and of 11β-hydroxylase was unchanged in zona fasciculata–reticularis cells from bromocriptine-treated rats.

There are several reports that dopamine D2 receptors could be detected in adrenal cortex cells. Amenta et al. have localized dopamine D2-like receptors in the zona glomerulosa, zona reticularis and zona fasciculata cells of the adrenal cortex, using autoradiography with light microscopy (Amenta et al., 1994). Furthermore, mRNA expression of dopamine D2 receptors was observed in zona glomerulosa, zona reticularis and, in a lesser amount, in zona fasciculata cells (Wu et al., 2001). The effects of bromocriptine treatment in vivo on corticosterone release might be partly a result of its direct effects. We further examined the direct effects of bromocriptine on corticosterone secretion. Although bromocriptine added in vitro did not affect the basal release of corticosterone, bromocriptine dose-dependently inhibited the corticosterone release in response to ACTH and forskolin. These data suggest that bromocriptine could acutely decrease the activity of adenylyl cyclase by acting directly on rat zona fasciculata–reticularis cells. Since the stimulatory effect of 8-Br-cAMP on corticosterone release was not altered by bromocriptine, it seemed that bromocriptine in situ did not affect the post-cAMP pathway.

In summary, the results demonstrated that bromocriptine in vivo inhibited the release of corticosterone via a mechanism involving inhibition of the response to ACTH, of the activity of adenylyl cyclase, of protein expression of StAR and P450scc, and of enzyme activity of P450scc in zona fasciculata–reticularis cells. Based on our in vitro results, the decreased corticosterone production was in part attributed to the inhibitory effect of bromocriptine on adenylyl cyclase acting directly on rat zona fasciculata–reticularis cells.

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