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Effect of Aging on Corticosterone Secretion in Diestrous Rats

Ming-Jae Lo,[1]* Mei-Mei Kau,[2] and Paulus S. Wang[3]

[1]Department of Early Childhood Education, National Tai-Chung Teachers College, Taichung, Taiwan, Republic of China
[2]National Taipei College of Nursing, Taipei, Taiwan, Republic of China
[3]Department of Physiology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China

Abstract The roles of age and prolactin (PRL) in regulating glucocorticoid secretion in diestrous rats were investigated. Adrenal zona fasciculata-reticularis (ZFR) cells from young, adult, middle (mid)-aged, and old female rats were isolated. Estrous cycle stage was determined by light microscopy after vaginal smears. Blood samples were collected from right jugular vein at 0, 30, 60, and 120 min after challenge with adrenocorticotropin (ACTH). During the diestrous phase, plasma levels of estradiol and progesterone were lower in mid-aged and old rats than in either young or adult rats. Age-dependent increases of the basal levels of plasma PRL and corticosterone were observed. No difference of ACTH-increased plasma concentrations of corticosterone was observed among young, adult, mid-aged, and old rats. Aging increased the basal, ACTH-, PRL-, forskolin (an adenylate cyclase activator)-, and 3-isobutyl-l-methylxanthine (IBMX, a non-selective phosphodiesterase inhibitor)-stimulated release of corticosterone and production of adenosine 3′, 5′-cyclic monophosphate (cAMP) in ZFR cells. However, the 8-Br-cAMP (a membrane-permeable cAMP)-stimulated release of corticosterone was not affected by age. Taken together, these data indicated that aging increased corticosterone secretion in female rats during diestrous phase, which is in part due to an increase in cAMP accumulation. In conclusion, aging and PRL play a stimulatory role in the co-regulation of corticosterone secretion. J. Cell. Biochem. 97: 351–358, 2006.

Key words: aging; diestrous; PRL; cAMP; corticosterone

Excessive glucocorticoids influence body composition, metabolism, cognition, and neuronal integrity [Lupien et al., 1999; Giordano et al., 2001; Rasmuson et al., 2002; Miller and O'Callaghan, 2003; Purnell et al., 2004]. Several studies report hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis in aged animals [Goncharova and Lapin, 2002; Lo and Wang, 2002]. However, studies investigating the effects of aging on the adrenal cortex present contradictory results [Cizza et al., 1994; Wang et al., 1997; Lo et al., 2000b].

It is well known that hyperprolactinemia occurs in aged animals [Lo et al., 1999, 2000b; Lu et al., 2000; Valdes Socin et al., 2002]. Although adrenocorticotropin (ACTH) is generally considered to play a major role in the regulation of adrenal glucocorticoid secretion, some reports suggest that other pituitary hormones (e.g., prolactin, PRL) also play a significant role in the regulation of adrenal function [Chang et al., 1999; Lo et al., 2000b; Lo and Wang, 2003; Silva et al., 2004]. The age-related corticosterone hypersecretion might be mediated via PRL, possibly through the change in the production of adenosine 3′, 5′-cyclic monophosphate (cAMP) [Lo and Wang, 2002, 2003].

Gonadal steroids can affect the stress-induced corticosterone release from the adrenal cortex [Lo et al., 2000a, 2004; Mitsushima et al., 2003]. Basal serum corticosterone concentrations are significantly greater in proestrus than

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*Correspondence to: Ming-Jae Lo, PhD, Department of Early Childhood Education, National Tai-Chung Teachers College, 140 Min-Shen Rd., Taichung 403, Taiwan, ROC.
E-mail: mjlo@ms3.ntctc.edu.tw

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in diestrous female rats [Mitsushima et al., 2003]. During aging in female rats, the estrous cycle ceases and the rats develop phases of constant estrous or constant diestrous prior to the irreversible transition into anestrous [Jarry et al., 1999]. Previous studies have examined the age-related changes of glucocorticoid secretion in males and ovariectomized females [Lo et al., 1999, 2000b; Lo and Wang, 2002]. However, the effect of age on adrenocortical function in gonadally-intact females is unknown.

The purpose of this study is to examine the effects of aging on the release of corticosterone in gonadally intact females, and to explore components of signal-transduction, that is, cAMP formation, in female rats during the diestrous phase and test for PRL involvement in the action mechanism.

MATERIALS AND METHODS

Animals

Young (3-month of age, n = 8), adult (6-month of age, n = 8), middle-aged (mid-aged; 12-month of age, n = 8), and old (22–24-month of age, n = 8) female rats of the Sprague–Dawley strain were used. They were housed in a temperature-controlled room ($22 \pm 1^{\circ}C$) with 14 h of artificial illumination daily (06:00–20:00). Food and water were given ad libitum.

In Vivo Experiments

Effects of aging on the concentrations of plasma estradiol, progesterone, and PRL in female rats during the diestrous phase. Vaginal smears were evaluated for determination of estrous cycle stage. Young, adult, mid-aged, and old female rats during the diestrous phase were used. Trunk blood was collected and plasma samples were withdrawn, then separated, and stored at $-20^{\circ}C$. The plasma levels of estradiol, progesterone, and PRL were assessed by radioimmunoassay (RIA).

Effects of aging on the concentrations of plasma corticosterone after a single intravenous (IV) injection of ACTH in female rats during the diestrous phase. All rats were anesthetized with ether and catheterized via the right jugular vein [Lo et al., 1998a,b]. Twenty hours following catheterization, rats were intravenously injected with ACTH (5 μg/ml/kg body weight). Blood samples (0.2 ml each) were collected from the jugular catheter at 0, 30, 60, and 120 min after challenge at 08:00. The lost blood volume was immediately replenished with heparinized saline after each sampling.

Preparation of plasma hormones for RIA. Plasma was separated by centrifugation at 10,000*g* for 1 min and stored at $-20^{\circ}C$. The concentrations of plasma PRL were measured by RIA. Plasma was mixed with diethyl ether ($10 \times$ vol), shaken for 20 min, centrifuged at 1,000*g* for 5 min, and then quickly frozen in a mixture of acetone and dry ice. The organic phase was collected, dried, and reconstituted in a buffer solution (0.1% gelatin in PBS, pH 7.5) before measuring the concentrations of estradiol, progesterone, and corticosterone by RIA.

In vitro Experiments

Preparation of zona fasciculata-reticularis (ZFR) cells for cell culture and incubation. Thirty-two rats, including young (n = 8), adult (n = 8), mid-aged (n = 8), and old rats (n = 8) were decapitated between 08:00–09:00 h, the preparation of ZFR cells for culture was performed following a method as previously described [Lo et al., 1998a,b].

The ZFR cells were incubated without (vehicle) or with hormones or agents dissolved in 1 ml per tube of Krebs–Ringer bicarbonate buffer [3.6 mmol K^+/L, 11.1 mmol glucose/L] with 0.2% bovine serum albumin (BSA) medium (KRBGA) for 30 min at 37°C under 95% O_2 and 5% CO_2. To measure the effects of aging on ACTH-, ovine prolactin (oPRL)-, forskolin-, 3-isobutyl-1-methylxanthine (IBMX)-, or 8-Br-cAMP-stimulated release of corticosterone or cAMP accumulation, rat ZFR cells were preincubated for 60 min with KRBGA. After preincubation, the cells were incubated in tubes containing 0.5 ml ACTH (10^{-8} and 10^{-7} M, Sigma Chemical Co., St. Louis, MO), oPRL (10^{-8} and 10^{-7} M, Sigma), forskolin (an adenylate cyclase activator, 10^{-6} and 10^{-5} M, Sigma), IBMX (a phosphodiesterase inhibitor, 5×10^{-4} and 5×10^{-3}M, Sigma), or 8-Br-cAMP (a membrane-permeable cAMP, 10^{-6}–10^{-4} M, Sigma) for 30 min. At the end of the incubation period, the cells were centrifuged at 200*g* at 4°C for 10 min. The supernatant fluid was stored at $-20^{\circ}C$ prior to analysis of corticosterone levels by RIA.

For studying the effects of aging on cAMP accumulation, cells were incubated with the medium containing ACTH (10^{-8} and 10^{-7} M),

oPRL (10^{-8} and 10^{-7} M), forskolin (10^{-6} and 10^{-5} M), or IBMX (5×10^{-4} and 5×10^{-3} M) for 30 min. At the end of the incubation period, cells were homogenized in 500 μl of 65% ice-cold ethanol, by polytron (PT-3,000, Kinematica Ag, Luzern, Switzerland) and centrifuged at 200*g* for 10 min. The supernatants were lyophilized in a vacuum concentrator (SpeedVac, Savant, Instruments, Holbrook, NY) and reconstituted with assay buffer (0.05M acetate buffer with 0.01% sodium azide, pH 6.2) before measurement of cAMP concentration by RIA.

RIA of estradiol. The concentrations of plasma estradiol were measured by RIA as previously described [Lu et al., 1998] with anti-estradiol serum (W-1). The sensitivity of estradiol RIA was 1 pg/ml. The intra- and interassay coefficients of variation were 5.3% (n = 5) and 4.2% (n = 5), respectively.

RIA of progesterone. Plasma progesterone concentrations were determined by RIA as described previously [Lu et al., 1996]. With anti-progesterone serum (W-5), the sensitivity of the progesterone RIA was 5 pg/ml. Intra- and interassay coefficients of variation were 7.3% (n = 4) and 4.9% (n = 5), respectively.

RIA of PRL. The concentrations of plasma PRL were measured by RIA as previously described [Wang et al., 1989]. The rat PRL-I-5 used for iodination and the PRL-RP-3 used as a standard preparation were provided by the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the U.S. Department of Agriculture, USA. The sensitivity of rat PRL RIA was 3 pg/ml. The intra- and interassay coefficients of variation were 4.1% (n = 7) and 5.1% (n = 5), respectively.

RIA of corticosterone. The concentrations of plasma and medium corticosterone were determined by RIA as described elsewhere [Chen et al., 1997; Lo et al., 1998a] with anti-corticosterone serum (PSW#4–9), the sensitivity of corticosterone RIA was 5 pg/ml. The intra- and interassay coefficients of variation were 2.8% (n = 8) and 3.6% (n = 7), respectively.

RIA of cAMP. The concentration of adrenal cAMP was determined by RIA as described elsewhere [Lu et al., 1996; Lo et al., 1998a] with anti-cAMP serum No. CV-27 pool. The sensitivity of cAMP RIA was 2 fmol/ml. The intra- and interassay coefficients of variation were 3.7% (n = 9) and 3.1% (n = 10), respectively.

Statistical Analysis

All data are expressed as mean values ± SEM. The treatment means values were tested for homogeneity by a two-way analysis and the difference between specific mean values was tested for significance using Duncan's multiple-range test [Steel and Torrie, 1981]. A difference between two mean values was considered statistically significant if P was less than 0.05.

RESULTS

Plasma Estradiol, Progesterone, and PRL

Both plasma estradiol and progesterone significantly decreased in mid-aged and old rats as compared with young and adult rats ($P < 0.05$ or $P < 0.01$, Fig. 1, top and center).

The basal levels of plasma PRL were significantly increased ($P < 0.01$) in mid-aged and old rats compared with either young or adult rats (Fig. 1, bottom).

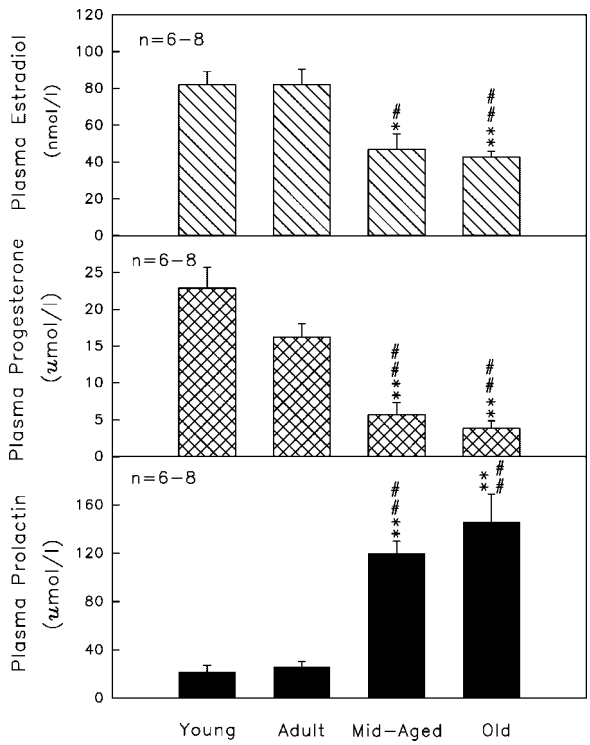

Fig. 1. Effects of aging on plasma estradiol (top), progesterone (center), and prolactin (bottom) concentrations (mean ± SEM) in young, adult, middle (mid)-aged, and old rats during the diestrous phase. Estrous cycle stage was determined by light microscopy of vaginal smears. Blood samples were collected from trunk after decapitation. *, **, $P < 0.05$, $P < 0.01$ as compared with young rats, respectively. #, ##, $P < 0.05$, $P < 0.01$ as compared with adult rats, respectively.

Plasma Corticosterone Concentrations After ACTH

The basal levels of plasma corticosterone significantly increased by 33% and 46% in mid-aged and old rats, respectively, as compared with young rats ($P < 0.05$ or $P < 0.01$, Fig. 2). Basal levels of plasma corticosterone were higher in old rats (92.4 ± 4.5 μmol/l, $P < 0.01$) than in adult rats (54.8 ± 9.9 μmol/l, Fig.2). However, no differences after IV injection of ACTH stimulated secretion of plasma corticosterone at 30 min were observed among age groups (Fig. 2).

A single IV injection of ACTH increased plasma concentrations of corticosterone at 30 min by 2.7-, 2.3-, 1.3-, and 1.2fold in young, adult, mid-aged, and old rats, respectively, compared with the basal level in the same group ($P < 0.05$ or $P < 0.01$, Fig. 2).

Compared to young rats, the levels of plasma corticosterone (blood samples collected at 120 min after challenge with ACTH) were lower in adult rats ($P < 0.05$, Fig. 2).

One hundred twenty minutes after injection of ACTH, plasma corticosterone levels were higher ($P < 0.01$) in old rats than in adult rats (Fig. 2).

Basal and ACTH-Stimulated Corticosterone Release and cAMP Production

The basal release of corticosterone by ZFR cells was greater in mid-aged ($P < 0.05$) and old rats ($P < 0.01$) than in young or adult rats (Fig. 3, top). Administration of ACTH (10^{-8} and 10^{-7} M) resulted in a dose-dependent increase of corticosterone release in young, adult, mid-aged, and old rats ($P < 0.01$, Fig. 3, top). The corticosterone release in response to ACTH (10^{-8} or 10^{-7} M) were markedly increased in ZFR cells from mid-aged or old rats compared with those from young and adult rats ($P < 0.01$, Fig. 3, top).

To further investigate the effects of age, we measured ACTH-induced intracellular cAMP production in rat ZFR cells from different ages (Fig. 3, bottom). The basal production of cAMP was significantly higher ($P < 0.05$ or $P < 0.01$) in mid-aged and old rats than in either young or adult rats (Fig. 3, bottom). The cAMP production in rat ZFR cells in response to ACTH (10^{-8} or 10^{-7} M) was increased ($P < 0.05$ or $P < 0.01$) in mid-aged or old rats compared with young or adult rats ($P < 0.05$ or $P < 0.01$, Fig. 3 bottom).

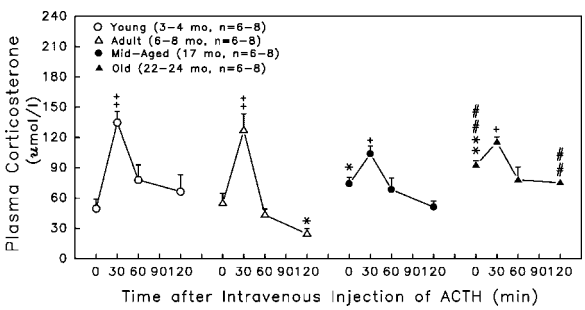

Fig. 2. Effects of a single IV injection of ACTH (5 μg/ml/kg body wt) in the morning (8 AM) on the plasma corticosterone concentrations (mean ± SEM) in young, adult, mid-aged or old female rats during the diestrous phase. Blood samples were collected through a right jugular catheter at times indicated. *, **, $P < 0.05$, $P < 0.01$ as compared with young rats, respectively. ##, $P < 0.01$ as compared with adult rats. +, ++, $P < 0.05$, $P < 0.01$ as compared with value at 0 min, respectively.

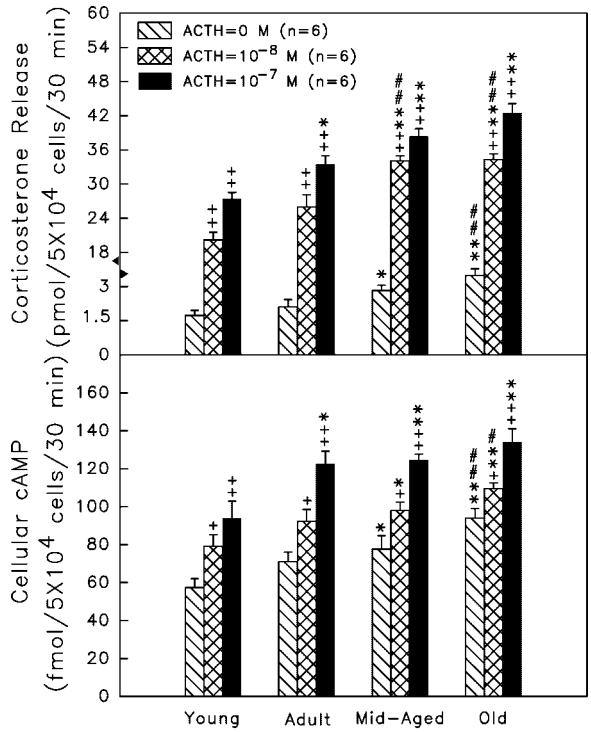

Fig. 3. Effects of aging on the basal (vehicle) and ACTH (10^{-8}, 10^{-7}M)-stimulated release of corticosterone (mean ± SEM) (top; please note discontinuous scales on the Y-axis) and adenosine 3′, 5′-cAMP production (mean ± SEM) (bottom) in ZFR cells from young, adult, mid-aged, or old rats during the diestrous phase. *, **, $P < 0.05$, $P < 0.01$ as compared with young rats, respectively. #, ##, $P < 0.05$, $P < 0.01$ as compared with adult rats, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with the vehicle group, respectively.

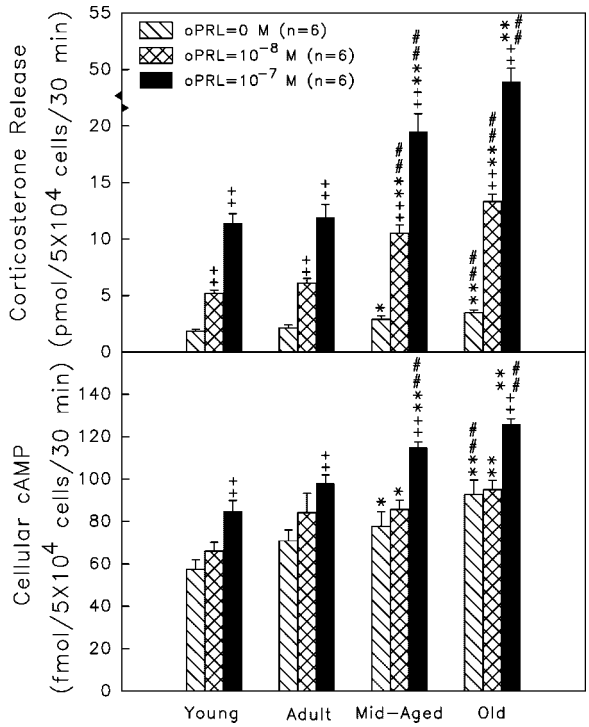

Fig. 4. Effects of aging on the basal (vehicle) and oPRL (10^{-8}, 10^{-7}M)-stimulated release of corticosterone (mean ± SEM) (top; please note discontinuous scales on the Y-axis) and cAMP production (mean ± SEM) (bottom) in ZFR cells from young, adult, mid-aged, or old rats during the diestrous phase. *, **, $P < 0.05$, $P < 0.01$ as compared with young rats, respectively. ##, $P < 0.01$ as compared with adult rats. +, ++, $P < 0.05$, $P < 0.01$ as compared with the vehicle group, respectively.

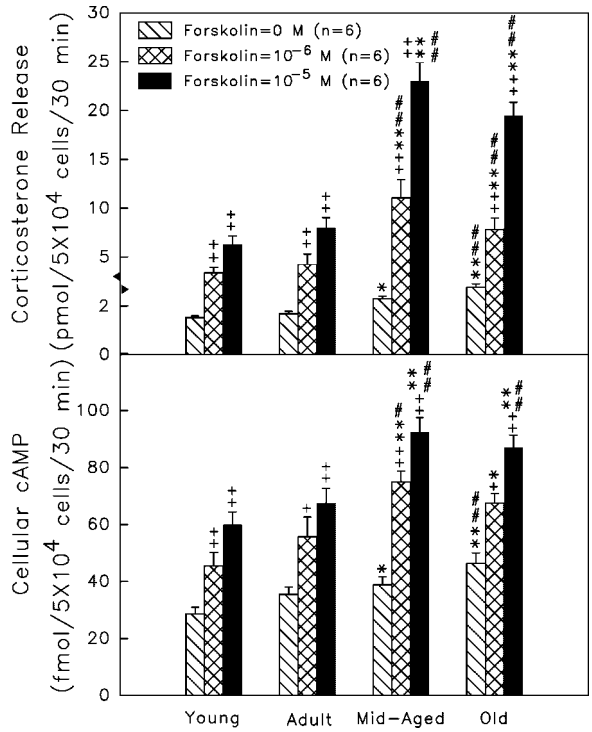

Fig. 5. Effects of aging on the basal (vehicle) and forskolin (10^{-6}, 10^{-5}M)-stimulated release of corticosterone (mean ± SEM) (top; please note discontinuous scales on the Y-axis) and cAMP production (mean ± SEM) (bottom) in ZFR cells from young, adult, mid-aged, or old rats during diestrous phase. *, **, $P < 0.05$, $P < 0.01$ as compared with young rats, respectively. #, ##, $P < 0.05$, $P < 0.01$ as compared with adult rats, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with the vehicle group, respectively.

oPRL-Stimulated Corticosterone Release and cAMP Production

Administration of oPRL (10^{-8} or 10^{-7} M) resulted in a dose-dependent increase of corticosterone release in all age groups ($P < 0.01$, Fig. 4, top). However, only the high dose of oPRL (10^{-7} M) increased the production of intracellular cAMP as compared to the basal level in the same age group ($P < 0.01$, Fig. 4, bottom). Age increased the release of corticosterone and production of intracellular cAMP in ZFR cells following incubation with either vehicle or oPRL (10^{-8} or 10^{-7} M) ($P < 0.05$ or $P < 0.01$, Fig. 4, top and bottom).

Forskolin-Stimulated Corticosterone Release and cAMP Production

Age enhanced the forskolin (10^{-6}, 10^{-5} M)-stimulated release of corticosterone and production of intracellular cAMP in ZFR cells as compared with either young or adult rats ($P < 0.05$ or $P < 0.01$, Fig. 5, top and bottom).

IBMX-Stimulated Corticosterone Release and cAMP Production

Administration of IBMX (5×10^{-4} and 5×10^{-3} M) resulted in an age-related increase in corticosterone release in ZFR cells ($P < 0.05$ or $P < 0.01$, Fig. 6, top).

The accumulation of cAMP in ZFR cells in response to either basal or IBMX (5×10^{-4} or 5×10^{-3} M) was higher ($P < 0.05$ or $P < 0.01$) in mid-aged and old rats than in young or adult rats (Fig. 6, bottom).

8-Br-cAMP-Stimulated Corticosterone Release

To evaluate the function of cAMP with aging, we examined the effect of exogenous administration of 8-Br-cAMP in ZFR cells from different age groups.

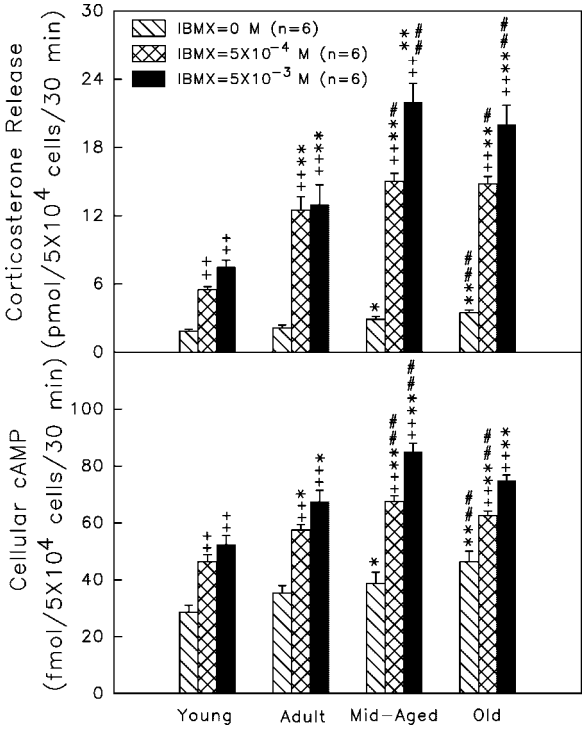

Fig. 6. Effects of aging on the basal (vehicle) and IBMX (5×10^{-4}, 5×10^{-3}M)-stimulated release of corticosterone (mean ± SEM) (top) and cAMP production (mean ± SEM) (bottom) in ZFR cells from young, adult, mid-aged, or old rats during the diestrous phase. *, **, $P < 0.05$, $P < 0.01$ as compared with young rats, respectively. #, ##, $P < 0.05$, $P < 0.01$ as compared with adult rats, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with the vehicle group, respectively.

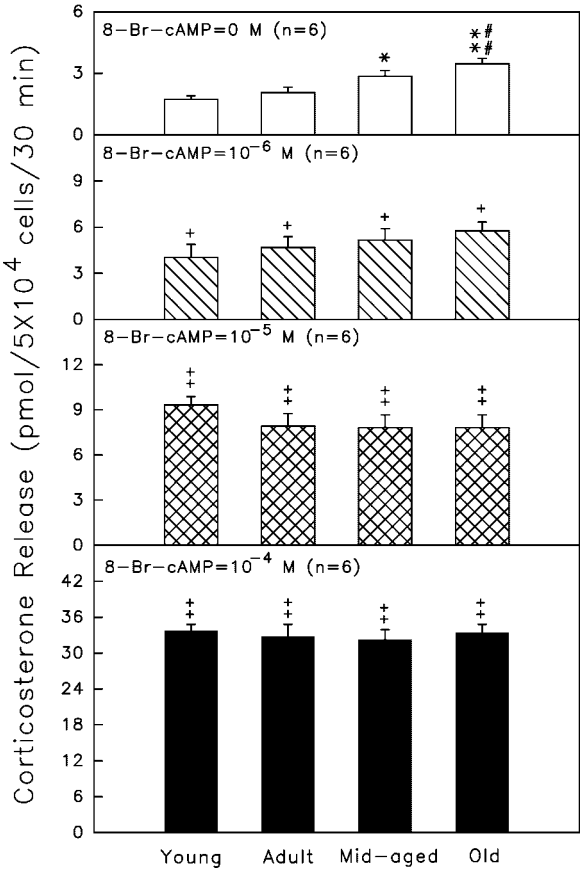

Fig. 7. Effects of aging on the basal (vehicle) and 8-Br-cAMP (10^{-6}–10^{-4}M)-stimulated release of corticosterone (mean ± SEM) in ZFR cells from young, adult, mid-aged, or old rats during the diestrous phase. *, **, $P < 0.05$, $P < 0.01$ as compared with young rats, respectively. ##, $P < 0.01$ as compared with adult rats. +, ++, $P < 0.05$, $P < 0.01$ as compared with the vehicle group, respectively.

Administration of 8-Br-cAMP (10^{-6}–10^{-4} M) for 30 min resulted in a concentration-dependent increase corticosterone release from ZFR cells as compared to the basal level in the same age group ($P < 0.05$ or $P < 0.01$, Fig. 7). However, with either low or high doses, no differences in 8-Br-cAMP-stimulated release of corticosterone were observed among young, adult, mid-aged, and old rats (Fig. 7).

DISCUSSION

Several studies report that the basal activity of the HPA axis tends to increase with age, often resulting in elevated secretion of glucocorticoids [Lupien et al., 1999, 2002; Lo and Wang, 2002; Carvalhaes-Neto et al., 2003; Zhao et al., 2003; Erwin et al., 2004]. Hypercortisolism and glucocorticoid feedback resistance might be general features of animal aging [Lo and Wang, 2002; Lupien et al., 2002; Carvalhaes-Neto et al., 2003; Miller and O'Callaghan, 2003; Erwin et al., 2004]. These observations agree with our in vivo and in vitro results, that is, aging enhanced the basal levels of corticosterone in diestrous rats.

The HPA axis in the female rat is most sensitive to stress during proestrous [Viau and Meaney, 1991]. Such enhanced HPA responses to stress are limited to the early portion of proestrous, as progesterone appears to inhibit the stimulatory effects of estrogen on ACTH release during stress [Viau and Meaney, 1991]. The HPA axis is modulated by progesterone or estrogen in rats [Viau and Meaney, 1991; Lo et al., 1999, 2000a; Lo and Wang, 2003]. In the present study, aging decreased the plasma levels of estradiol and progesterone but increased the corticosterone concentration in diestrous rats. However, the precise mechanisms remain undefined at the present time.

Cortisol secretion in response to corticotropin-releasing hormone (CRH) is higher in old female monkeys compared with young monkeys [Goncharova and Lapin, 2002]. However, the ACTH depot test reveals no age-related changes in the maximum capacity of monkey adrenals to synthesize and secrete cortisol [Goncharova and Lapin, 2002]. Our data are consistent with these findings because we found no differences in peak plasma corticosterone.

It is well established that ACTH stimulates corticosterone secretion both in vivo and in vitro, via a mechanism dependent on cAMP generation [Lo et al., 1998a; Chang et al., 2002, 2004; Lo and Wang, 2002]. We found that as compared with either young or adult rats, mid-aged and old female rats also show high corticosterone release in response to ACTH from the ZFR cells, which is in agreement with previous observations [Lo et al., 1999; Lo and Wang, 2002]. The stimulant effect of ACTH on cAMP generation in female rat ZFR cells is enhanced by age. Forskolin, the adenylate cyclase activator, significantly increased the release of corticosterone or cAMP production and potentiated the aging effect. IBMX, a non-selective phosphodiesterase inhibitor, significantly increased the release of corticosterone and cAMP production. Age increased the IBMX-induced corticosterone release and cAMP production in rat ZFR cells. A membrane-permeable cAMP (8-Br-cAMP) increased the release of corticosterone in all age groups, and effectively attenuated the age differences in corticosterone secretion. These results confirm our previous finding and suggest that age-increased corticosterone production in rats during diestrous phase is due in part to an increase of cAMP generation.

It is well known that hyperprolactinemia occurs in older animals and activates the HPA axis [Lo et al., 1999, 2000b; Lu et al., 2000; Valdes Socin et al., 2002], which is in agreement with the present finding. The increased corticosterone secretion during hyperprolactinemia might be related to the increased secretion of ACTH, as well as a direct effect of PRL on adrenal steroidogenesis [Chang et al., 1999; Lo et al., 2000b; Lo and Wang, 2003; Silva et al., 2004]. The increase in adrenal weight and the area of zona fasciculata cells observed in hyperprolactinemic rats may be due to PRL-induced adrenocortical cell hypertrophy [Silva et al., 2004]. It has been shown that chronic hyperprolactinemia enhances the receptor-G-protein-adenylate cyclase coupling and cAMP production [Chang et al., 1999]. It has been indicated that the cellular cAMP and calcium influx might be involved in the secondary messenger-signaling pathway of oPRL on corticosterone secretion [Chang et al., 1999; Lo et al., 2000b; Lo and Wang, 2003]. In the present study, only the high dose of oPRL increased the production of intracellular cAMP. We, therefore, suggest that oPRL-stimulated release of corticosterone is due in part to cAMP-independent pathways, for example, the calcium pathway. Our results indicated an age-dependent increase of corticosterone release and production of intracellular cAMP in ZFR cells following incubation with oPRL. This evidence suggested that due to aging as well as to hyperprolactinemia the adrenal cortex could be more sensitive to PRL stimulation resulting in higher cAMP production compared to young animals. In summary, our data suggest that hypersecretion of corticosterone in response to aging is due in part to increased cAMP production and enhanced stimulatory effect of PRL in ZFR cells of gonadally intact female rats during the diestrous phase.

ACKNOWLEDGMENTS

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