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Involvement of cAMP but not PKA in the Increase of Corticosterone Secretion in Rat Zona Fasciculata-Reticularis Cells by Aging

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Abstract The effects and mechanisms of aging on corticosterone secretion in zona fasciculata-reticularis (ZFR) cells of ovariectomized (Ovx) rats were studied. Young (3-month) and old (24-month) female rats were Ovx for 4 days before decapitation. ZFR cells were isolated and incubated with different hormones or reagents at 37°C for 30 min. Aging increased the basal secretion of corticosterone both in vivo and in vitro. The adrenocorticotropin (ACTH)-, forskolin-, 3-isobutyl-l-methylxanthine (IBMX)-, 8-bromo-adenosine 3′,5′-cyclic monophosphate (8-Br-cAMP)-, and ovine prolactin (oPRL)-stimulated release of corticosterone by ZFR cells was greater in old than in young Ovx rats. H89, an inhibitor of protein kinase A (PKA), decreased the production of corticosterone in ZFR cells from young but not old Ovx rats. Forskolin-, or IBMX-induced production of cAMP was greater in old than in young Ovx animals, which correlated with the increase of corticosterone production by aging. The activity of 11β-hydroxylase that converts deoxycorticosterone (DOC, 10^{-9} or 10^{-8} M) to corticosterone in rat ZFR cells was decreased by age. However, the corticosterone production in response to high dose of DOC (10^{-7} M) was indifferent between young and old groups. These results suggest that aging increases corticosterone production in Ovx rats via a mechanism in part associated with an increase of adenylyl cyclase activity and a decrease of phosphodiesterase activity, and then an increase of the generation of cAMP, but not related to either PKA activity or 11β-hydroxylase. J. Cell. Biochem. 85: 35–41, 2002.

Key words: cAMP; ZFR cells; Ovx rat; PKA activity

Aging is commonly associated with a dysregulation and functional impairment of the hypothalamic-pituitary-adrenal (HPA) axis [Slotkin et al., 1998; Hassan et al., 1999]. In humans, aging is accompanied by an increase of adrenal glucocorticoid secretion [Yen and Laughlin, 1998]. Elderly males have impaired cellular immunity and are more predisposed to opportunistic infections after long-term glucocorticoid treatment [Tornatore et al., 1998]. Corticosterone, the major glucocorticoid in the rat, induces hippocampus neuronal death in old animals [Herbert, 1998]. These studies reflect that the decline of many physiological functions in aged animals is closely correlated with the change of glucocorticoid secretion.

It has been shown that estradiol receptors localized in brain regions may mediate HPA function [Rainbow et al., 1982]. Ovariectomy decreases the synthesis and release of adrenocorticotropin (ACTH) and corticosterone [Kitay, 1963; Coyne and Kitay, 1969]. Estrogen treatment has been shown to alter ACTH and corticosterone at multiple levels [Burgess and Handa, 1992]. Since the HPA axis is modulated by estrogen in women and rats [Coyne and Kitay, 1969; Handa et al., 1994; Burleson et al., 1998; De-Leo et al., 1998; Lo et al., 1999, 2000], the ovariectomized (Ovx) rats were used to observe the effect of aging on corticosterone secretion in the present study.

Hyperprolactinemia has been observed in old rats [Bolzan et al., 1995; Mohankumar et al.,

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1997]. The activation of HPA axis observed during the course of hyperprolactinemia might be explained by a direct stimulatory effect of prolactin (PRL) on HPA axis [Eldridge and Lymangrover, 1984; Albertson et al., 1987; Weber and Calogero, 1991; Glasow et al., 1996].

It has been well known that protein kinase A (PKA) is an enzyme which phosphorylates some cellular proteins and can be activated by adenosine 3′,5′-cyclic monophosphate (cAMP) following tropic hormone stimulation [Popplewell et al., 1986]. Although numerous studies have examined age-related changes of glucocorticoid secretion [Wang et al., 1997; Yen and Laughlin, 1998; Lo et al., 1999], the role of adenylyl cyclase-cAMP/PKA-dependent signaling pathway in the regulation of adrenocortical function during aging is still unclear. This study was undertaken to evaluate the effect of aging on the secretion of corticosterone, and the possible correlations between corticosterone secretion and cAMP production, PKA activity, or the 11β-hydroxylase activity of steroidogenesis during aging.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats of 3-month (young), and 24-month (old) of age were ovariectomized (Ovx) 4 days prior to experimentation. 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. All animal experimentation has been conducted humanely and in conformance with the policy statement of the Committee of National Yang-Ming University.

Effects of Age on the Concentrations of Plasma Corticosterone in Ovx Rats

Ovariectomy was performed under ether anesthesia. Four days post-ovariectomy 12-young and 12-old rats were then decapitated between 08:00–09:00, trunk blood was collected and plasma samples were withdrawn, then separated and stored at -20°C. The concentration of corticosterone in plasma was measured by radioimmunoassay (RIA) [Lo et al., 1998a,b].

Preparation of Zona Fasciculata-Reticularis (ZFR) Cells for Cell Culture

An adrenocortical preparation enriched with ZFR cells for culture was performed following a method as previously described [Lo et al., 1998a,b].

The ZFR cells were incubated with or without hormones or agents dissolved in 1 ml/tube of Krebs-Ringer bicarbonate buffer with 3.6 mmol K^+/L, 11.1 mmol glucose/L and 0.2% BSA (KRBGA medium) for 30 min at 37°C under 95% O_2 and 5% CO_2. For studying the effect of aging on the activity of adenylyl cyclase, phosphodiesterase, and PKA, as well as accumulation of cAMP, cells were incubated for 30 min with the medium containing ACTH (1×10^{-9} and 1×10^{-8} M), forskolin (an adenylyl cyclase activator, 1×10^{-6} and 1×10^{-5} M), 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase inhibitor, 5×10^{-5} and 50×10^{-5} M), 8-Br-cAMP (a membrane-permeable cAMP analogue, 5×10^{-5}, 10×10^{-5} M), H 89 (a PKA inhibitor, $1 \times 10^{-9} \sim 1 \times 10^{-7}$ M), and ovine prolactin (oPRL, $1 \times 10^{-8} \sim 1 \times 10^{-6}$ M). The medium was collected, the cells from KRBGA, ACTH, forskolin, IBMX, oPRL group were homogenized in 500 μl of 65% ice-cold ethanol, by polytron (PT-3000, 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 (0.05 M sodium acetate buffer with 0.01% sodium azide, pH 6.2) before measuring the concentration of cAMP by RIA.

To observe the effects of age on 11β-hydroxylase activities, ZFR cells were pre-incubated for 60 min with KRBGA medium. After pre-incubation, the cells were incubated in tubes containing 0.5 ml deoxycorticosterone (DOC, $1 \times 10^{-9} \sim 1 \times 10^{-7}$ M) for 60 min.

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/assay tube. The intra- and interassay coefficients of variation were 4.2% ($n = 7$) and 4.7% ($n = 9$), respectively.

RIA of cAMP

The concentration of adrenal cAMP determined by RIA as described elsewhere [Lo et al., 1998a]. With anti-cAMP serum No. CV-27 pool, the sensitivity of cAMP was 2 fmol/assay tube. The intra- and interassay coefficients of

variation were 6.3% ($n = 4$) and 6.9% ($n = 5$), respectively.

Statistical Analysis

All data were expressed as mean ± SEM. The treatment means were tested for homogeneity using an ANOVA, and the difference between specific means was tested for significance using Duncan's multiple-range test [Steel and Torrie, 1960]. A difference between two means was considered statistically significant when P was less than 0.05 or 0.01.

Materials

ACTH, forskolin, IBMX, 8-Br-cAMP, H89, ovine PRL, and DOC were provided by Sigma (St. Louis, MO). ^{3}H-corticosterone was from Amersham (UK). The anti-cAMP serum CV-27 pool was a gift from NIH, USA.

RESULTS

Aging Effects on Basal and oPRL-Induced Corticosterone Release and cAMP Production

There were age-, and dose-dependent increases of the oPRL ($1 \times 10^{-8} \sim 1 \times 10^{-6}$ M)-stimulated release of corticosterone and cAMP production in Ovx rat ZFR cells ($P < 0.05$ or 0.01, Fig. 1, top and bottom).

Aging Effects on Basal and ACTH-Induced Corticosterone Release and cAMP Production in ZFR Cells

Compared to young Ovx rats, the basal release of corticosterone in ZFR cells after incubation for 30 min increased significantly ($P < 0.01$) by 1.4-fold in old Ovx rats (Fig. 2, top).

Administration of ACTH (1×10^{-9} or 1×10^{-8} M) for 30 min markedly increased the corticosterone release in ZFR cells by 23 ~ 37-folds ($P < 0.01$) (Fig. 2, top).

Old Ovx rats showed a greater increase of corticosterone release in ZFR cells ($P < 0.05$ or 0.01) following incubation with ACTH as compared with young Ovx rats (Fig. 2, top).

There were age-related increases (37 ~ 46%, $P < 0.01$) of the basal and ACTH-stimulated cAMP accumulations in Ovx rat ZFR cells (Fig. 2, bottom).

Aging Effects on Forskolin-Induced Corticosterone Release and cAMP Production

Administration of forskolin (1×10^{-6} or 1×10^{-5} M) in vitro for 30 min resulted in a

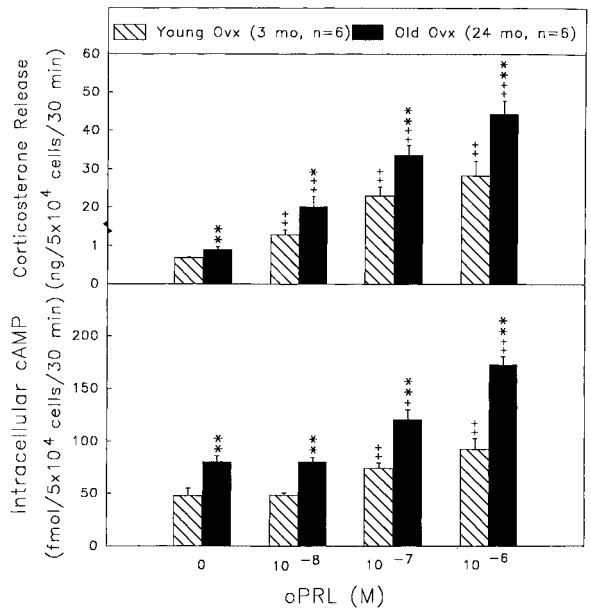

Fig. 1. Effects of aging on the basal and oPRL ($10^{-8} \sim 10^{-6}$ M)-stimulated release of corticosterone (top) and cAMP production (bottom) for 30 min from ZFR cells of ovariectomized (Ovx) rat in vitro. Female rats were Ovx for 4 days before decapitation. *, **, $P < 0.05$, $P < 0.01$ as compared with young Ovx rats, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with oPRL = 0 M, respectively. Each value represents the mean ± SEM.

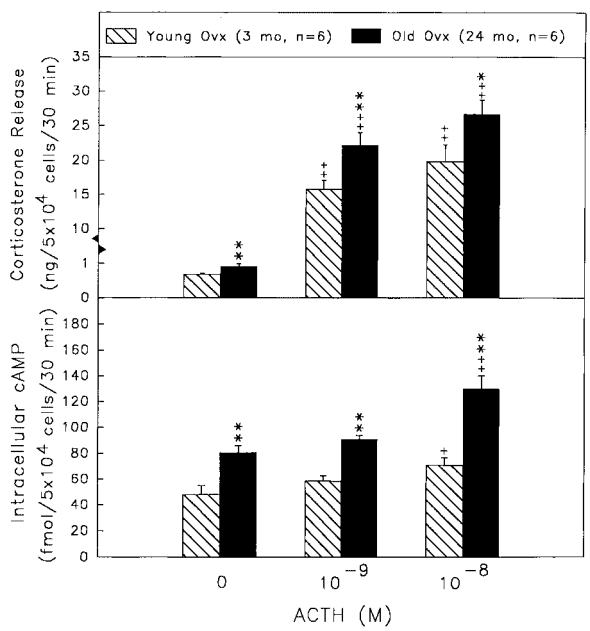

Fig. 2. Effects of aging on the basal and ACTH (10^{-9}, 10^{-8} M)-stimulated release of corticosterone (top) and cAMP production (bottom) for 30 min from ZFR cells of Ovx rat in vitro. *, **, $P < 0.05$, $P < 0.01$ as compared with young Ovx rats, respectively. +, ++, $P < 0.05$, $P < 0.01$ as compared with ACTH = 0 M, respectively. Each value represents the mean ± SEM.

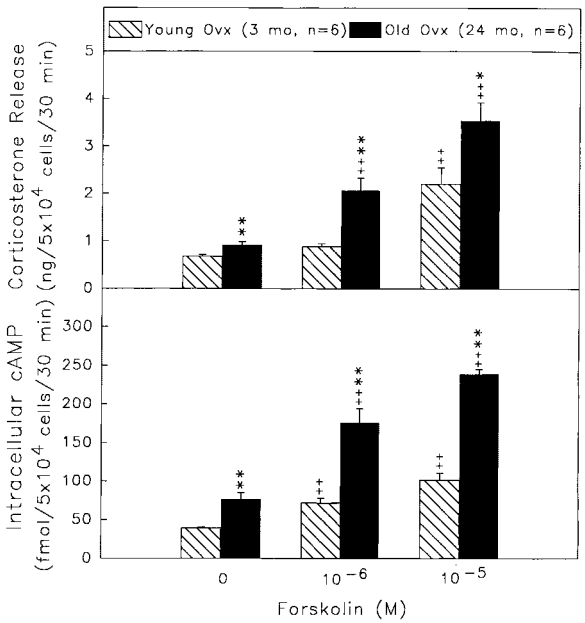

Fig. 3. Effects of aging on the corticosterone release (top) and cAMP production (bottom) after incubation of Ovx rat ZFR cells with forskolin (10^{-6}, 10^{-5} M) for 30 min. *, **, $P<0.05$, $P<0.01$ as compared with young Ovx rats, respectively. +, ++, $P<0.05$, $P<0.01$ as compared with forskolin = 0 M, respectively. Each value represents the mean ± SEM.

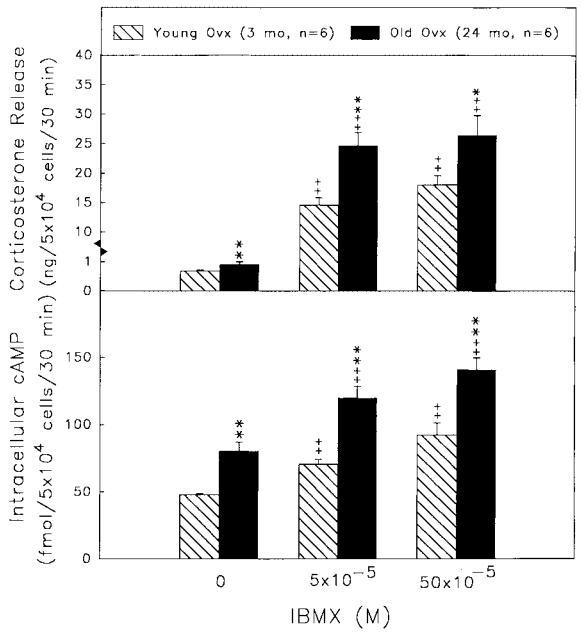

Fig. 4. Effects of aging on corticosterone release (top) and cAMP production (bottom) after incubation of Ovx rat ZFR cells with IBMX (5×10^{-5}, 50×10^{-5} M) for 30 min. *, **, $P<0.05$, $P<0.01$ as compared with the young Ovx rats, respectively. ++, $P<0.01$ as compared with the group treated with IBMX = 0 M. Each value represents the mean ± SEM.

greater increase of the corticosterone release and intracellular cAMP production in old Ovx rat ZFR cells as compared with young Ovx rats ($P<0.05$ or 0.01, Fig. 3, top and bottom).

Aging Effects on Corticosterone Release and cAMP Production in Response to IBMX

The levels of corticosterone and intracellular cAMP after incubation of rat ZFR cells with IBMX are shown in Figure 4. There were age-related increases of corticosterone release by $47\sim70\%$ ($P<0.05$ or 0.01, Fig. 4, top) and cAMP production by $53\sim70\%$ ($P<0.01$, Fig. 4, bottom) following incubation of rat ZFR cells with IBMX (5×10^{-5} or 50×10^{-5} M) for 30 min.

Aging Effects on 8-Br-cAMP Induced Corticosterone Release

Incubation of 8-Br-cAMP (5×10^{-5} or 10×10^{-5} M) for 30 min resulted in greater release of corticosterone ($P<0.01$) by ZFR cells in old Ovx rats than in young Ovx rats (Fig. 5).

Aging Effects on Corticosterone Release in Response to H89

Incubation of H89 (a blocker of PKA) with ZFR cells for 30 min dose-dependently decreased the release of corticosterone in young Ovx rats, but not in old Ovx rats (Fig. 6). The corticosterone production in response to H89 ($1\times10^{-9}\sim1\times10^{-7}$ M) was greater ($2.3\sim2.9$-fold) in old than in young groups.

Aging Effects on Corticosterone Release in Response to Deoxycorticosterone

The increase of corticosterone production in response to 1×10^{-9} M or 1×10^{-8} M DOC was

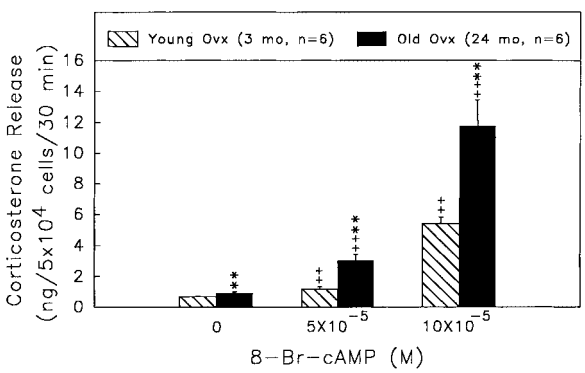

Fig. 5. Effects of aging on 8-Br-cAMP (5×10^{-5}, 10×10^{-5} M)-stimulated release of corticosterone for 30 min from ZFR cells of Ovx rat in vitro. **, $P<0.01$ as compared with young Ovx rats. ++, $P<0.01$ as compared with 8-Br-cAMP = 0 M. Each value represents the mean ± SEM.

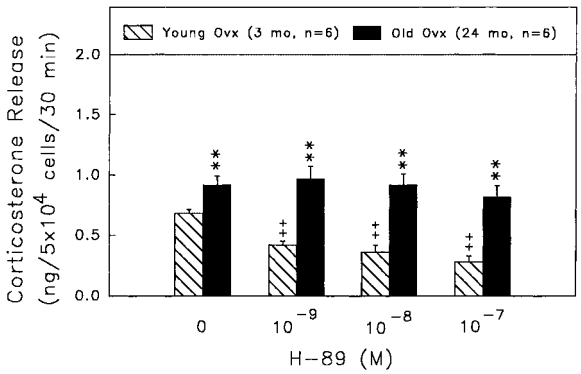

Fig. 6. Effects of aging on the release of corticosterone in response to H 89 ($10^{-9} \sim 10^{-7}$ M) for 30 min from ZFR cells of Ovx rat in vitro. **, $P < 0.01$ as compared with young Ovx rats. ++, $P < 0.01$ as compared with H 89 = 0 M. Each value represents the mean ± SEM.

attenuated by age ($P < 0.01$, Fig. 7). The corticosterone production in response to high dose of DOC (1×10^{-7} M) was indifferent between young and old groups.

DISCUSSION

It has been well established that a decline in neuroendocrine functions occurred during aging [Kizaki et al., 1998]. There are still controversies concerning the effect of aging on the basal level of plasma glucocorticoid in humans [Van Kampen and Fuchs, 1998; Yen and Laughlin, 1998]. In elderly humans, hypercortisolism may cause hippocampal damage and impair hippocampus-dependent learning and memory [Lupien et al., 1998]. It has been shown that tropic hormone-stimulated corticosterone production in isolated adrenocortical cells was

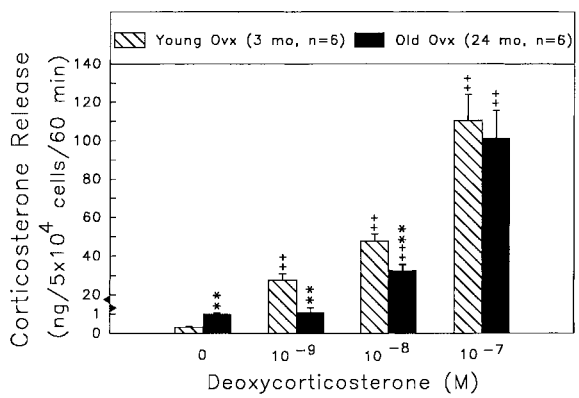

Fig. 7. Effects of aging on deoxycorticosterone (DOC, $10^{-9} \sim 10^{-7}$ M)-stimulated release of corticosterone for 60 min from ZFR cells of Ovx rat in vitro. **, $P < 0.01$ as compared with young Ovx rats. ++, $P < 0.01$ as compared with vehicle. Each value represents the mean ± SEM.

declined by age [Azhar and Reaven, 1994]. However, many human or rat studies reported an increase of glucocorticoid production with age [Sapolsky and Altmann, 1991; Lupien et al., 1998; Lo et al., 1999, 2000]. These findings suggest that hypercortisolism and glucocorticoid feedback resistance might be the general features of animal aging [Sapolsky and Altmann, 1991; Lupien et al., 1998]. Our in vivo and in vitro studies have shown that in old Ovx rats, the basal levels of plasma (young Ovx rats vs. old Ovx rats is 86.1 ± 8.9 ng/ml vs. 157.8 ± 13.5 ng/ml; data not shown) and medium corticosterone increased as compared with young Ovx rats.

Aging and hypercortisolism may be associated with alterations of stress-induced hormone release [Gotthardt et al., 1995]. Our in vitro study indicates that ACTH-stimulated release of corticosterone and cAMP production for 30 min were higher in old than in young Ovx rats. These results are in agreement with our previous reports that aging increases the ACTH-stimulated release of corticosterone in oil- or estradiol-replaced Ovx rats (3-month vs. 12-month) [Lo et al., 1999].

We examined the effects of aging on agonist-stimulated (e.g., ACTH, forskolin, 8-Br-cAMP) corticosterone and cAMP production in Ovx rat ZFR cells (Figs. 2, 3, and 5), and found that aging enhances the stimulatory effect of ACTH on intracellular cAMP production in Ovx rats. The reason might be due to an increased number of ACTH binding sites or binding affinity in rat adrenocortical cells by age [Popplewell et al., 1986]. Our results indicated that forskolin (an adenylyl cyclase activator) or IBMX (a phosphodiesterase inhibitor)-stimulated release of corticosterone and cAMP production in old Ovx rats was still higher than in young Ovx animals. It seems that the activity of adenylyl cyclase and phosphodiesterase might be increased and decreased, respectively, in Ovx rat ZFR cells during aging. The mechanism underlying these changes is likely to be multifactors, but may be in part due to increased activity of adenylyl cyclase and decreased cAMP catabolism in ZFR cells by aging.

In the present study, exogenous 8-Br-cAMP (a membrane-permeable cAMP analogue; 5×10^{-5} or 10×10^{-5} M) was unable to amplify the steroid production in young Ovx rats to same level found in old Ovx rats. We suggest that the activation of the post-cAMP pathway

certainly plays an important role in the increase of corticosterone release in old Ovx rats. Administration of H89 (a PKA inhibitor) was unable to inhibit the production of corticosterone in old Ovx rats. These studies reflect the alteration of corticosterone secretion by aging is via the adenylyl cyclase-cAMP signaling pathway and other pathway (e.g., Ca^{2+} or cGMP) rather than the activation of PKA.

PRL receptor in the adrenal gland has been reported in rats, guinea pigs, and human beings [Sautin et al., 1989; Outhit et al., 1993; Nagano and Kelly, 1994; Glasow et al., 1996]. It has been shown that PRL has a direct effect on adrenal steroidogenesis, thereby regulating adrenal function [Albertson et al., 1987; Glasow et al., 1996]. Recently, we have demonstrated that PRL increases the production of corticosterone by acting directly on rat ZFR cells via cAMP cascades and 3β HSD enzyme activity [Chang et al., 1999; Lo et al., 1999]. Our in vitro study indicates that PRL-stimulated release of corticosterone and cAMP production in old Ovx rats were higher than in young Ovx rats. Our present in vitro study confirms that PRL stimulated the release of corticosterone by ZFR cells, which is correlated with age-related increase of plasma corticosterone in rats. These data suggest that the direct and stimulatory effect of PRL on corticosterone release in part via an increase of cAMP production in ZFR cells.

Steroidogenic acute regulatory protein (StAR) is essential for adrenal steroidogenesis, which participate in steroidogenesis through the mitochondrial transfer of cholesterol to cytochrome P450scc [Ariyoshi et al., 1998; Hasegawa et al., 2000]. In older rats ($18 \sim 20$-month-old), the lipoprotein uptake pathway appears to be intact in adrenals, but the intracellular processing of internalized cholesteryl ester is defective [Azhar and Reaven, 1994]. In the present study, we evaluated the possible correlation between corticosterone secretion and the post-pregnenolone steroid enzyme activity on age-increased steroidogenesis in Ovx rats by using DOC, the substrate of 11β-hydroxylase. Inasmuch as exogenous administration of a higher dose of DOC was able to enhance corticosterone production in young rats to the corticosterone level in old rats, we suggest that the 11β-hydroxylase activity is not altered by advancing age.

In summary, these findings suggest that the hypersecretion of corticosterone induced by aging in Ovx rats is in part due to: (1) the increase of adenylyl cyclase activity, (2) the decrease of phosphodiesterase activity, (3) the increased generation or accumulation of cAMP, but not due to the activities of PKA and 11β-hydroxylase. The increased production of cAMP may cause some PKA-independent crosstalk to increase corticosterone production.

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REFERENCES

Albertson BD, Sienkiewicz ML, Kimball D, Munabi AK, Cassorla F, Lyn N, Loriaux D. 1987. New evidence for a direct effect of prolactin on rat adrenal steroidogenesis. Endocr Res 13:317–333.

Ariyoshi N, Kim YC, Artemenko I, Bhattacharyya KK, Jefcoate CR. 1998. Characterization of the rat Star gene that encodes the predominant 3.5-kilobase pair mRNA. ACTH stimulation of adrenal steroids in vivo precedes elevation of Star mRNA and protein. J Biol Chem 273:7610–7619.

Azhar S, Reaven E. 1994. Effect of age on cholesterol uptake and utilization by rat adrenals: Internalization of lipoprotein-derived cholesteryl esters. Mech Ageing 77: 13–25.

Bolzan AD, Brown OA, Goya RG, Bianchi MS. 1995. Hormonal modulation of antioxidant enzyme activities in young and old rats. Exp Gerontol 30:169–175.

Burgess LH, Handa RJ. 1992. Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone secretion, and glucocorticoid receptor mediated function in female rats. Endocrinology 131:1261–1269.

Burleson MH, Malarkey WB, Cacioppo JT, Poehlmann KM, Kiecolt-Glaser JK, Berntson GG, Glaser R. 1998. Postmenopausal hormone replacement: Effects on autonomic, neuroendocrine, and immune reactivity to brief psychological stressors. Psychosom Med 60:17–25.

Chang LL, Lo MJ, Kan SF, Huang WJS, Chen JJ, Kau MM, Wang JL, Lin H, Tsai SC, Chaio YC, Yeh JY, Wun WSA, Wang PS. 1999. Direct effects of prolactin on corticosterone release by zona fasciculata-reticularis cells from male rats. J Cell Biochem 73:563–572.

Chen YH, Lo MJ, Kau MM, Tsai SC, Chiao YC, Chen JJ, Liaw C, Lu CC, Lee BP, Chen SC, Fang VS, Ho LT, Wang PS. 1997. Inhibition of corticosterone secretion by thyroxine in male rats. Chinese J Physiol 40:25–30.

Coyne MD, Kitay JI. 1969. Effect of ovariectomy on pituitary secretion of ACTH. Endocrinology 85:1097–1102.

De-Leo V, la-Marca A, Talluri B, D'Antona D, Morgante G. 1998. Hypothalamo-pituitary-adrenal axis and adrenal function before and after ovariectomy in premenopausal women. Eur J Endocrinol 138:430–435.

Eldridge JC, Lymangrover JR. 1984. Prolactin stimulates and potentiates adrenal steroid secretion in vitro. Horm Res 20:252–260.

Glasow A, Breidert M, Haidan A, Anderegg U, Kelly PA, Bornstein SR. 1996. Functional aspects of the effect of prolactin (PRL) on adrenal steroidogenesis and distribution of the PRL receptor in the human adrenal gland. J Clin Endocrinol Metab 81:3103–3111.

Gotthardt U, Schweiger U, Fahrenberg J, Lauer CJ, Holsboer F, Heuser I. 1995. Cortisol, ACTH, and cardiovascular response to a cognitive challenge paradigm in aging and depression. Am Physiol 268:R865–R873.

Handa RJ, Burgess LH, Kerr JE, O'Keefe JA. 1994. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. Horm Behav 28:454–476.

Hasegawa T, Zhao LH, Caron KM, Majdic G, Suzuki T, Shizawa S, Sasano H, Parker KL. 2000. Developmental roles of the steroidogenic acute regulatory protein (StAR) as revealed by StAR knockout mice. Mol Endocrinol 14:1462–1471.

Hassan AH, Patchev VK, von Rosenstiel P, Holsboer F, Almeida OF. 1999. Plasticity of hippocampal corticosteroid receptors during aging in the rat. FASEB J 13:115–122.

Herbert J. 1998. Neurosteroids, brain damage, and mental illness. Exp Gerontol 33:713–727.

Kitay JI. 1963. Pituitary-adrenal function in the rat after gonadectomy and gonadal hormone replacement. Endocrinology 73:253–260.

Kizaki T, Ookawara T, Oh-Ishi S, Itoh Y, Iwabuchi K, Onoe K, Day NK, Good RA, Ohno H. 1998. An increase in basal glucocorticoid concentration with age induces suppressor macrophages with high-density Fc gamma RII/III. Immunology 93:409–414.

Lo MJ, Kau MM, Chen YH, Tsai SC, Chiao YC, Chen JJ, Liaw C, Lu CC, Lee BP, Chen SC, Fang VS, Ho LT, Wang PS. 1998a. Acute effects of thyroid hormones on the production of adrenal cAMP and corticosterone in male rats. Am J Physiol 274:E238–E245.

Lo MJ, Wang SW, Kau MM, Chen JJ, Chen YH, Fang VS, Ho LT, Wang PS. 1998b. Pharmacological effects of propylthiouracil on corticosterone secretion in male rats. J Investig Med 46:444–452.

Lo MJ, Chang LL, Wang PS. 1999. Age-related differences in corticosterone secretion in female rats. Metabolism 48:535–541.

Lo MJ, Kau MM, Cho WL, Wang PS. 2000. Aging effects on the secretion of corticosterone in male rats. J Investig Med 48:335–342.

Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, Thakur M, McEwen BS, Hauger RL, Meaney MJ. 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat Neurosci 1:69–73.

Mohankumar PS, Mohankumar SM, Quadri SK, Voogt JL. 1997. Chronic hyperprolactinemia and changes in dopamine neurons. Brain Res Bull 42:435–441.

Nagano M, Kelly PA. 1994. Tissue distribution and regulation of rat prolactin receptor gene expression. Quantitative analysis by polymerase chain reaction. J Biol Chem 269:13337–13345.

Outhit A, Morel G, Kelly PA. 1993. Visualization of gene expression of short and long forms of prolactin receptor in the rat. Endocrinology 133:135–147.

Popplewell PY, Tsubokawa M, Ramachandran J, Azhar S. 1986. Differential effects of aging on adrenocorticotropin receptors, adenosine 3′5′-monophosphate response, and corticosterone secretion in adrenocortical cells from Sprague-Dawley rats. Endocrinology 119:2206–2213.

Rainbow TC, Parsons B, MacLusky NJ, McEwen BS. 1982. Estradiol receptor levels in rat hypothalamic and limbic nuclei. J Neurosci 2:1439–1445.

Sapolsky RM, Altmann J. 1991. Incidence of hypercortisolism and dexamethasone resistance increases with age among wild baboons. Biol Psychiatry 30:1008–1016.

Sautin II, Tron'ko ND, Mikosha AS. 1989. Receptor of prolactin and its effect on binding of corticotropin in human and guinea pig adrenocortical cells. Biull Eksp Biol Med 108:177–179.

Slotkin TA, Seidler FJ, Ritchie JC. 1998. Effects of aging and glucocorticoid treatment on monoamine oxidase subtypes in rat cerebral cortex: Therapeutic implications. Brain Res Bull 47:345–348.

Steel RGD, Torrie JH. 1960. Principles and procedures of statistics. McGraw-Hill New York.

Tornatore KM, Venuto RC, Logue G, Davis PJ. 1998. CD^{4+} and CD^{8+} lymphocyte and cortisol response patterns in elderly and young males after methylprednisolone exposure. J Med 29:159–183.

Van Kampen M, Fuchs E. 1998. Age-related levels of urinary free cortisol in the tree shrew. Neurobiol Aging 19:363–366.

Wang PS, Lo MJ, Kau MM. 1997. Glucocorticoids and aging. J Formos Med Assoc 96:792–801.

Weber RFA, Calogero AE. 1991. Prolactin stimulates rat hypothalamic corticotropin-releasing hormone and pituitary adrenocorticotropin secretion in vitro. Neuroendocrinology 54:248–253.

Yen SS, Laughlin GA. 1998. Aging and the adrenal cortex. Exp Gerontol 33:897–910.