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# Differential Involvement of Protein Kinase C in Basal Versus Acetylcholine-Regulated Prolactin Secretion in Rat Anterior Pituitary Cells During Aging

**Hsiao-Fung Pu and Tsuei-Chu Liu***

Department of Physiology, School of Medicine, National Yang-Ming University, Shih-Pai, Taipei 112, Taiwan, Republic of China

**Abstract** Although it is well known that plasma concentration of prolactin (PRL) increases during aging in rats, how the anterior pituitary (AP) aging per se affects PRL secretion remains obscure. The objectives of this study were to determine if changes in the pituitary PRL responsiveness to acetylcholine (ACh; a paracrine factor in the AP), as compared with that to other PRL stimulators or inhibitors, contribute to the known age-related increase in PRL secretion, and if protein kinase C (PKC) is involved. We also determined if replenishment with aging-declined hormones such as estrogen/thyroid hormone influences the aging-caused effects on pituitary PRL responses. AP cells were prepared from old (23–24-month-old) as well as young (2–3-month-old) ovariectomized rats. Cells were pretreated for 5 days with diluent or 17β-estradiol ($E_2$; 0.6 nM) in combination with or without triiodothyronine ($T_3$; 10 nM). Then, cells were incubated for 20 min with thyrotropin-releasing hormone (TRH; 100 nM), angiotensin II (AII; 0.2–20 nM), vasoactive intestinal peptide (VIP; $10^{-9}$–$10^{-5}$ M), dopamine (DA; $10^{-9}$–$10^{-5}$ M), or ACh ($10^{-7}$–$10^{-3}$ M). Cells were also challenged with ACh, TRH, or phorbol 12-myristate 13-acetate (PMA; $10^{-6}$ M) following PKC depletion by prolonged PMA ($10^{-6}$ M for 24 h) pretreatment. We found that estrogen priming of AP cells could reverse the aging-caused effects on pituitary PRL responses to AII and DA. In hormone-replenished cells aging enhanced the stimulation of PRL secretion by TRH and PMA, but not by AII and VIP. Aging also reduced the responsiveness of cells to ACh and DA in suppressing basal PRL secretion, and attenuated ACh inhibition of TRH-induced PRL secretion. Furthermore, ACh suppressed TRH-induced PRL secretion mainly via the PMA-sensitive PKC in the old AP cells, but via additional mechanisms in young AP cells. On the contrary, basal PRL secretion was PKC (PMA-sensitive)-independent in the old AP cells, but dependent in the young AP cells. Taken together, these results suggest differential roles of PMA-sensitive PKC in regulating basal and ACh-regulated PRL responses in old versus young AP cells. The persistent aging-induced differences in AP cell responsiveness to ACh, DA, TRH, and PMA following hormone ($E_2$/$T_3$) replenishment suggest an intrinsic pituitary change that may contribute, in part, to the elevated in vivo PRL secretion observed in aged rats. J. Cell. Biochem. 86: 268–276, 2002. 

**Key words:** rat pituitary cells; aging; acetylcholine; protein kinase C; 17β-estradiol; triiodothyronine; thyrotropin-releasing hormone; angiotensin II; dopamine

Age-related increases in prolactin (PRL) gene expression and secretion as well as in the percent of lactotropes have been reported in the rat anterior pituitary (AP) [Chuknyiska et al., 1986; Larson and Wise, 1991; Rossi et al., 1993; Tan et al., 1997]. Plasma and pituitary concentrations of PRL are elevated in old as compared with young rats [Haji et al., 1984; Tan et al., 1997]. Increases in plasma PRL concentrations first become detectable in middle-aged cycling and non-cycling rats, and continue to increase with aging [Wise, 1982, 1984; Demarest et al., 1985]. How the aging of AP per se affects PRL secretion remains obscure.

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*Correspondence to: Tsuei-Chu Liu, Department of Physiology, National Yang-Ming University, Shih-Pai, Taipei 112, Taiwan, Republic of China. E-mail: hfpu@ym.edu.tw

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Secretion of PRL by the AP is mainly under the hypothalamic dopamine (DA) inhibition. It is also stimulated by hypothalamic thyrotropin-releasing hormone (TRH). Other inhibitory and stimulatory factors synthesized in the pituitary and hormones present in the circulation (i.e., estrogens and thyroid hormones) also affect PRL secretion [Maurer, 1982; Carmeliet et al., 1989; Dymshitz et al., 1992; Ray and Melmed, 1997; Bruhn et al., 1998; Cai et al., 1998; Kanyicska et al., 1998; Pu et al., 1999]. Among the autocrine/paracrine factors in the pituitary, angiotensin II (AII) and vasoactive intestinal peptide (VIP) have been shown to stimulate, and acetylcholine (ACh) to inhibit PRL secretion [Rudnick and Dannies, 1981; Carmeliet et al., 1989; Balsa et al., 1996; Díaz-Torga et al., 1998; Pu et al., 1999]. Recently, we have further demonstrated that the suppression by ACh of both basal and TRH-induced PRL secretion was enhanced in a 17β-estradiol ($E_2$)- and triiodothyronine ($T_3$)-dependent manner in AP cells prepared from ovariectomized young rats [Pu et al., 1999]. Furthermore, multiple intracellular pathways including nitric oxide are differentially involved in ACh actions on these two PRL responses in AP cells [Pu et al., 1999]. In this article, we investigated the mechanisms involved in aging-enhanced PRL secretion. We studied if changes in the pituitary responsiveness to ACh, as compared with that to AII, VIP, or DA, contribute to the known aging-related increase in PRL secretion, and if protein kinase C (PKC) is involved. We also determined if replenishment with aging-declined hormones such as estrogen/thyroid hormone [Mariotti et al., 1995; Lamberts et al., 1997] influences the aging-caused effects on pituitary PRL responses. Our data revealed persistent aging-induced differences in AP cell responsiveness to ACh, DA, TRH, and phorbol 12-myristate 13-acetate (PMA) following hormone ($E_2/T_3$) replenishment. This suggests an intrinsic pituitary change that may contribute, in part, to the elevated in vivo PRL secretion observed in aged rats. Furthermore, PMA-sensitive PKC appears to be differentially involved in basal as opposed to ACh-regulated PRL secretion during pituitary aging.

## MATERIALS AND METHODS

### Animals and Pituitary Cell Cultures

Young cycling (2–3 months) and old diestrous (23–24 months) female Sprague-Dawley rats were purchased from National Yang-Ming University Animal Center. They were maintained under temperature ($22 \pm 2^{\circ}C$) and light (lights on: 0600–2000 h) controlled conditions with free access to food and water. Rats were bilaterally ovariectomized under ether anesthesia and decapitated by a guillotine 4 days later. Old rats with visible, enlarged, and hemorrhagic pituitary tumors were not included in the studies. Anterior pituitaries (APs) were excised, dispersed into single cell suspension as described previously [Liu and Jackson, 1987]. The tissue fragments were sliced and dissociated by collagenase and hyaluronidase after a brief exposure to trypsin. Routinely, the dispersed cells were cultured ($2 \times 10^5$ cells/ml/well) in 24-well plate (Falcon, Lincoln Park, NJ) overnight at $37^{\circ}C$ under moist 5% $CO_2$ and 95% air. The culture medium contained 2.5% FBS (Hyclone Laboratories, Logan, Utah) and 10% bovine calf serum (Hyclone) in supplemented medium-199 without phenol red (weak estrogen). All sera were pretreated with dextran-charcoal to remove small molecules including steroids and thyroid hormones [Liu et al., 1993].

### Incubation With Test Drugs

As specified in each experiment, overnight cultured AP cells were treated for 3 days with either $E_2$ (Sigma, St. Louis, MO; 0.6 nM), or $E_2$ plus $T_3$ (Sigma; 10 nM). Then cells were washed and incubated at $37^{\circ}C$ in sera-free medium containing 1% BSA (Sigma) and initial hormone treatments for additional 2 days, with medium changed each day. Subsequently, cells were washed, then challenged at $37^{\circ}C$ for 20 min with PRL regulators in the sera-free medium containing 1% BSA without hormone treatments. Cells were also pretreated with phorbol 12-myristate 13-acetate (PMA, $10^{-6}$ M, a PKC activator) for 24 h to deplete PKC before challenge with PRL regulators. At the end of incubation, the medium was collected, centrifuged, and stored at $-20^{\circ}C$ for measuring PRL by RIA. The PRL regulators used included ACh ($10^{-7}$, $10^{-5}$, $10^{-3}$ M), TRH (100 nM), PMA ($10^{-6}$ M), AII (0.2, 2, 20 nM), DA ($10^{-9}$, $10^{-7}$, $10^{-5}$ M), and VIP ($10^{-9}$, $10^{-7}$, $10^{-5}$ M), all obtained from Sigma.

### RIA for PRL

PRL RIA kit, kindly provided by the National Hormone and Pituitary Program of NIDDK (Torrance, CA) and Dr. Parlow, was used to

measure PRL in the medium. The standard was rPRL-RP-3 (lot no. AFP-4459B), the iodinated PRL was rPRL-I6 (lot no. AFP-10505B), and the antibody was anti-rPRL-IC-5 (lot no. AFP425-10-91). The sensitivity of the assay was 30 pg/tube/ml assay volume. The intra- and inter-assay coefficients of variation were 9.0 and 14.5%, respectively [Tan et al., 1997]. Data were expressed as nanogram PRL/well/20 min.

### Experimental Design and Statistical Analysis

In each experiment, approximately 20 APs were pooled for the preparation of one batch of AP cells. Aliquots of each cell batch were placed in separate wells, and drug treatments were randomly assigned to each well. Each experiment was replicated at least three times. The effects of drugs on PRL secretion were expressed as fold of control (without drug) in respective old or young cells. Due to heterogeneity of error [Tan et al., 1997], data were subjected to logarithmic transformation before statistical analysis by one way ANOVA using the SAS system (SAS Institute, Inc., Cary, NC). Multiple comparisons were performed according to the Fisher's least significant difference (LSD) test, where ANOVA was significant [Steel and Torrie, 1980]. All data were expressed as mean ± SEM. Differences between means were considered significant when $P < 0.05$, and highly significant when $P < 0.01$.

## RESULTS

### Effects of Aging on AII- and VIP-Enhanced PRL Secretion

These experiments were performed in AP cells pretreated with or without $E_2$ for 5 days before challenge with AII and VIP for 20 min. Both AII (0.2, 2, 20 nM; Fig. 1) and VIP ($10^{-9}$, $10^{-7}$, $10^{-5}$ M; Fig. 2) dose-dependently stimulated PRL secretion in AP cells from either young or old rats, regardless of $E_2$ priming. However, in the absence of $E_2$ (Fig. 1, left panel), the PRL releasing abilities for all three doses of AII were significantly attenuated ($P <$ at least 0.05) in the old as compared with the young AP cells. By contrast, in the presence of $E_2$ (Fig. 1, right panel), the differences between the two groups of cells were completely abolished. For VIP-stimulated PRL secretion, there were no significant differences at the three VIP doses between the two age groups of cells, either in the absence of $E_2$ (Fig. 2, left panel), or in the presence of $E_2$ (Fig. 2, right panel).

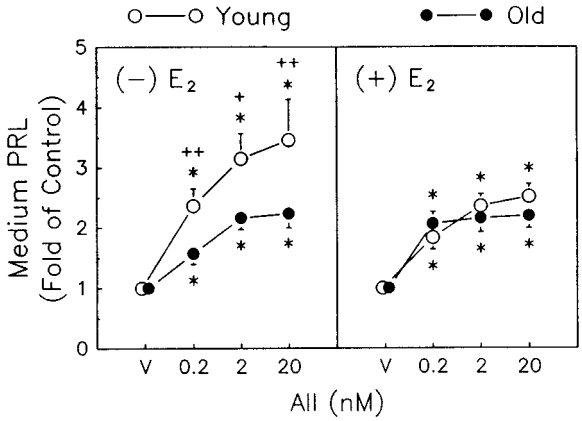


**Fig. 1.** Effect of aging on angiotensin II (AII)-enhanced PRL secretion. Anterior pituitary (AP) cells ($2 \times 10^5$ cells/ml/well) prepared from 4-day ovariectomized young or old rats were cultured for 5 days in the presence or absence of 0.6 nM $E_2$ as described in Materials and Methods. Then cells were washed and challenged for 20 min without (vehicle; V) or with AII (0.2, 2, 20 nM) in sera-free medium containing 1% BSA in the absence of $E_2$. Vehicle-treated young or old cells, with or without $E_2$-priming, serve as controls for respective AII-treated cells. Data are expressed as fold of respective controls. Each point represents the mean ± SEM of three experiments with triplicate cultures per drug treatment per experiment. $^*P <$ at least 0.05: AII versus respective control; $^+P < 0.05$, $^{++}P < 0.01$: old versus respective young.

### Effect of Aging on DA-Suppressed PRL Secretion

AP cells were pretreated with or without $E_2$ before challenge with DA ($10^{-9}$, $10^{-7}$, $10^{-5}$ M).

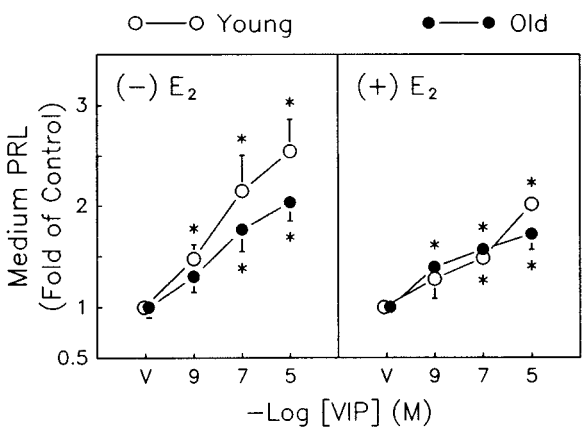


**Fig. 2.** Effect of aging on VIP-enhanced PRL secretion. See legend to Figure 1. AP cells were pretreated with or without $E_2$ for 5 days. At the end of culture, cells were washed and then challenged for 20 min without (vehicle; V) or with VIP ($10^{-9}$, $10^{-7}$, $10^{-5}$ M) in sera-free medium containing 1% BSA in the absence of $E_2$. Each point represents the mean ± SEM of three experiments with triplicate cultures per drug treatment per experiment. $^*P <$ at least 0.05: VIP versus respective control.

DA dose-dependently suppressed PRL secretion in AP cells from either young or old rats, irrespective of $E_2$ (Fig. 3). However, in the absence of $E_2$ (Fig. 3, left panel), the suppression by DA at both $10^{-7}$ and $10^{-5}$ M on PRL secretion was greater ($P < 0.01$) in the old than in the young AP cells. Interestingly, $E_2$ pretreatment caused the AP cells to become less ($P < 0.05$) responsive to $10^{-7}$ M DA in the old as compared with the young cells (Fig. 3, right panel).

### Effect of Aging on $E_2$-Stimulated PRL Secretion

For young AP cells, basal PRL secretion in $E_2$-unprimed and $E_2$-primed cells were $18.3 \pm 1.0$ and $39.6 \pm 1.7$ ng/well/20 min ($n = 9$), respectively. The fold stimulation by $E_2$ was $2.2 \pm 0.2$ ($P < 0.01$). For old AP cells, basal PRL secretion in $E_2$-unprimed and $E_2$-primed cells were $37.9 \pm 2.1$ and $66.4 \pm 2.3$ ng/well/20 min ($n = 9$), respectively. The fold stimulation by $E_2$ was $1.7 \pm 0.1$ ($P < 0.05$). There was no significant difference on fold of $E_2$ stimulation between the old and the young AP cells.

### Effect of Aging on ACh-Suppressed PRL Secretion

The action of ACh on PRL secretion was examined in the absence (Fig. 4, left panel) or presence (Fig. 4, right panel) of TRH in $E_2 + T_3$ pretreated AP cells [Pu et al., 1999]. ACh ($10^{-7}$, $10^{-5}$, $10^{-3}$ M) dose-dependently inhibited both basal (Fig. 4, left panel) and TRH-induced PRL secretion (Fig. 4, right panel), regardless of aging. However, the sensitivity to low ACh dose ($10^{-7}$ M) for suppressing basal PRL secretion was significantly less ($P < 0.05$) in the old as compared with the young AP cells. Furthermore, the maximal suppression by a high ACh dose ($10^{-3}$ M) on TRH-induced PRL secretion was also significantly attenuated ($P < 0.01$) in the old as opposed to the young AP cells.

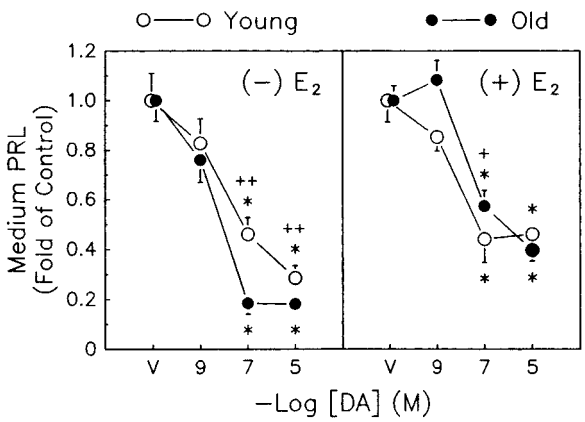


**Fig. 3.** Effect of aging on DA-suppressed PRL secretion. See legend to Figure 1. AP cells were pretreated with or without $E_2$ for 5 days. At the end of culture, cells were washed and then challenged for 20 min without (vehicle; V) or with DA ($10^{-9}$, $10^{-7}$, $10^{-5}$ M) in sera-free medium containing 1% BSA in the absence of $E_2$. Each point represents the mean ± SEM of three experiments with triplicate cultures per drug treatment per experiment. *$P <$ at least 0.05: DA versus respective control; $^{+}P < 0.05$, $^{++}P < 0.01$ old versus. respective young.

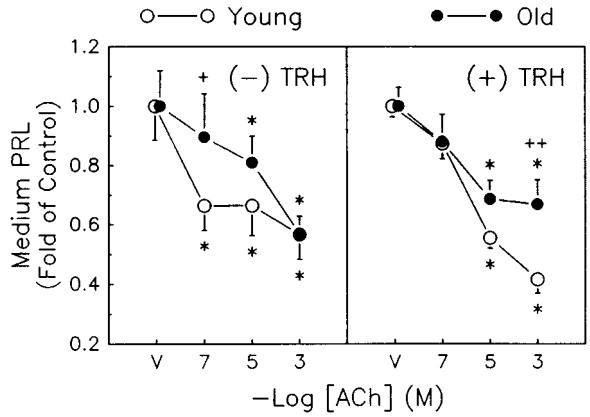


**Fig. 4.** Effect of aging on ACh-suppressed PRL secretion. All AP cells were pretreated with 0.6 nM $E_2$ plus 10 nM $T_3$ for 5 days. Then, cells were washed and treated for 20 min without (vehicle; V) or with ACh ($10^{-7}$, $10^{-5}$, $10^{-3}$ M) in the presence or absence of TRH (100 nM) in sera-free medium containing 1% BSA without $E_2/T_3$. Vehicle-treated young or old cells, with or without TRH stimulation, serve as controls for respective ACh-treated cells. Data are expressed as fold of respective controls. Each point represents the mean ± SEM of three experiments with triplicate cultures per drug treatment per experiment. *$P <$ at least 0.05: ACh versus respective control; $^{+}P < 0.05$, $^{++}P < 0.01$: old versus respective young.

### Effects of Aging on TRH- and PMA-Stimulated PRL Secretion

AP cells were pretreated with $E_2 + T_3$, and then challenged with or without TRH or PMA for 20 min. The magnitude of basal PRL secretion in old AP cells was approximately 1.9-fold of that in young cells ($118.4 \pm 14.1$ vs. $61.2 \pm 6.9$ ng/well/20 min, respectively, $n = 6$, $P < 0.01$), an observation similar to our previous studies [Tan et al., 1997]. The fold stimulation on basal PRL secretion in response to a maximum dose of TRH ($10^{-7}$ M) was significantly greater in the old than in the young AP cells ($4.0 \pm 0.4$ vs. $2.4 \pm 0.3$ fold, respectively, $n = 6$, $P < 0.01$; Fig. 5B). Likewise, the fold stimulation in response to PMA ($10^{-6}$ M) on basal PRL secretion was significantly higher in the old than in

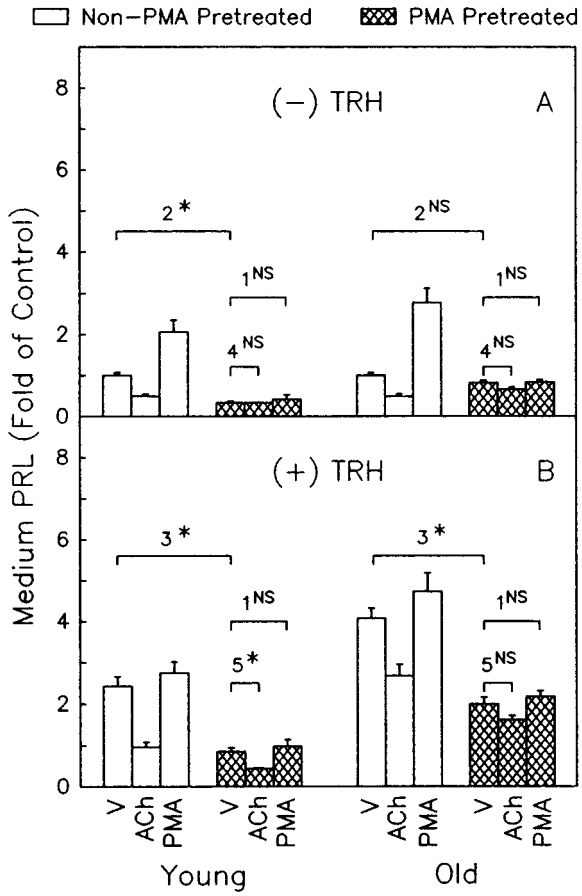


**Fig. 5.** Effects of PMA pretreatment on PRL secretion in old versus young AP cells. AP cells cultured in medium containing 0.6 nM $E_2$ plus 10 nM $T_3$ for 5 days were pretreated with PMA ($10^{-6}$ M) during the last 24 h incubation period to deplete PKC. Then, cells were washed and challenged for 20 min without any drug (vehicle; V), or with ACh ($10^{-3}$ M) or PMA ($10^{-6}$ M) in the absence (**panel A**) or presence (**panel B**) of TRH ($10^{-7}$ M). Non-PMA pretreated young or old cells challenged with vehicle in the absence of TRH serve as controls. Values are expressed as fold of respective controls. Each bar represents the mean ± SEM of three to six experiments with triplicate cultures per drug treatment per experiment. Statistical analyses between the bracketed two groups are shown as NS (not significant); $^*P < 0.01$. Numbers above the brackets refer to the comparisons made in Results.

the young AP cells ($2.7 \pm 0.2$ vs. $2.1 \pm 0.2$ fold, respectively, $n = 6$, $P < 0.01$; Fig. 5A).

### Effects of PKC Depletion on Basal, TRH-Stimulated and ACh-Suppressed PRL Secretion in Old Versus Young AP Cells

$E_2 + T_3$ cultured old or young AP cells were pretreated with PMA ($10^{-6}$ M) for 24 h to deplete cellular PMA-sensitive PKC. Then cells were challenged with either ACh ($10^{-3}$ M) or PMA ($10^{-6}$ M) in the absence (Fig. 5A) or presence (Fig. 5B) of TRH ($10^{-7}$ M) for 20 min. In PMA-pretreated cells, the second challenge dose of PMA was unable to elicit PRL secretion, regardless of age or TRH (Fig. 5 A,B, comparison 1), confirming the depletion of PMA-sensitive PKC. Notably, PKC depletion reduced basal PRL secretion in the young ($P < 0.01$) AP cells, but not in the old ones (Fig. 5A, comparison 2). PKC depletion also decreased ($P < 0.01$) the TRH-induced PRL secretion in both young and old AP cells (Fig. 5B, comparison 3); however, the percent decrease was greater in the young than in the old AP cells ($61.3 \pm 4.7\%$ vs. $51.6 \pm 1.7\%$, respectively, $P < 0.01$). Nevertheless, both young and old cells remained responsive to TRH under PKC depletion conditions (Fig. 5; vehicle group in panel B vs. respective vehicle group in panel A; $P < 0.01$). We further found that PKC depletion blocked ACh suppression of basal PRL secretion in either young or old AP cells (Fig. 5A, comparison 4), but eliminated ACh suppression of TRH-induced PRL secretion only in the old AP cells (Fig. 5B, comparison 5). The fold of ACh suppression in the young and old AP cells were $46.2 \pm 6.2\%$ vs. $17.9 \pm 8.0\%$, respectively ($P < 0.01$).

## DISCUSSION

We have demonstrated that in hormone ($E_2 + T_3$ or $E_2$) replenished AP cells aging attenuated ACh- and DA-inhibited PRL secretion. Aging also enhanced PRL secretion induced by either TRH or PMA but not by AII or VIP. Furthermore, aging altered basal PRL secretion from PKC (PMA-sensitive)-dependent manner to the PKC (PMA-sensitive)-independent manner. Aging downregulated multiple intracellular pathways responsible for ACh suppression of TRH-induced PRL secretion to the PKC (PMA-sensitive)-dependent pathway only. The persistent aging-induced differences in PRL responses of AP cells following hormone replenishment suggest an intrinsic pituitary change that may contribute, in part, to the elevated in vivo PRL secretion observed in aged rats.

The aging-attenuated ACh suppression of PRL secretion as well as the differential involvement of PMA-sensitive PKC in aging-caused changes of basal PRL secretion versus ACh-suppressed PRL secretion, to our knowledge, has not been reported previously. We noted that following hormone replenishment with $E_2 + T_3$, ACh at $10^{-7}$ M suppressed basal PRL secretion

in the young but not in the old AP cells. Furthermore, a maximal dose of ACh at $10^{-3}$ M reduced the fold of TRH-stimulated PRL secretion more in the young than in the old AP cells. These findings suggest an aging-induced decline in PRL responses to ACh. We also found that depletion of PMA-sensitive PKC by pretreatment of cells with $10^{-6}$ M PMA for 24 h abolished the suppression of basal PRL secretion by ACh, regardless of age. This observation indicates that in both age groups the PMA-sensitive PKC plays a critical role in the ACh-suppressed basal PRL secretion. PKC depletion also abrogated the suppression by ACh ($10^{-3}$ M) on TRH-induced PRL secretion in the old but not in the young AP cells. This finding demonstrates the importance of PMA-sensitive PKC on ACh suppression of TRH-stimulated PRL secretion in the old rather than in the young AP cells. It appears that in the old AP cells ACh suppresses TRH-induced PRL secretion mainly via the PKC (PMA-sensitive) pathway. Other intracellular signals such as the cAMP/$Ca^{2+}$ pathways, and the nitric oxide pathway essential for ACh-regulated PRL secretion in the young AP cells [Pu et al., 1999] may be down-regulated in the aged AP cells. Consistent with this observation, reduction in pituitary phosphotidylinositol turnover and cAMP/cAMP binding protein has been found in aged as opposed to young rats [Arima, 1982; Tang and Tang, 1983; Bonetti et al., 1987]. The aging-induced intracellular signaling changes, together with aging-decreased maximal binding sites for pituitary high affinity muscarinic receptors [Avissar et al., 1981] may explain the reduced efficacy of ACh on inhibiting PRL secretion in old AP cells. Additionally, we demonstrated that PKC depletion greatly decreased basal PRL secretion in the young but not in the old-AP cells. The PKC (PMA-sensitive) pathway appears to play an indispensable role in the young-AP cells, as opposed to a dispensable role in the aged AP cells, for the maintenance of basal PRL secretion, suggesting the involvement of other mechanisms. Nitric oxide has been shown to suppress basal PRL secretion and is involved in ACh-regulated PRL secretion [McCann et al., 1998; Pu et al., 1999]. Recently, we have observed that the aging-related increase in pituitary PRL secretion was accompanied by the declined nitric oxide production in aged AP cells (unpublished data). This finding seems to be consistent with the hypothesis that nitric oxide may cause pituitary aging and affects secretion of hormones by AP [McCann et al., 1998].

Our data revealed that aging-altered PRL response to DA was influenced by the availability of $E_2$. Following in vitro $E_2$ replenishment the fold of DA ($10^{-7}$ M) suppression on PRL secretion was less in the old than in the young-AP cells. However, in the absence of $E_2$, DA at both $10^{-7}$ and $10^{-5}$ M reduced basal PRL secretion more effectively in the old than in the young AP cells. Increased PRL responses to DA observed in $E_2$-unprimed old AP cells as opposed to young AP cells may reflect the lack of long term in vivo $E_2$ stimulation in the old AP cells rather than aging of the pituitary per se. On the other hand, aging-induced reduction in PRL responses to DA noted in $E_2$-primed AP cells may be attributed to intrinsic changes of pituitary during aging. Larson and Wise [1991], using AP cells prepared from ovariectomized cycling old and young rats pretreated with or without $E_2$ capsule for 4 days, similarly demonstrated that aging attenuated the inhibitory effect of DA on PRL secretion by individual lactotropes. $E_2$ has been known to augment PRL gene expression [Stone et al., 1977; Maurer, 1982; Borgundvaag et al., 1992; Watters et al., 2000], the percentage of cells secreting PRL [Larson and Wise, 1991], the intracellular $Ca^{2+}$ concentration [Shin et al., 1993], and the PKC isozyme expression and total activity [Maeda and Lloyd, 1993]. $E_2$ also reduced DA receptor [Kochman et al., 1989; Nedvidkova et al., 2001], DA-inhibited adenylyl cyclase activity as well as the expression of inhibitory G-proteins ($G_{i\alpha 3}/G_o$) in lactotropes [Borgundvaag and George, 1988; Maus et al., 1989; Livingstone et al., 1998]. Although we did not observe significant effect of aging on basal PRL secretion stimulated by $E_2$ alone, all of these $E_2$-regulated events may affect pituitary responsiveness to DA. How aging alters specific pathways relevant to DA-inhibition of PRL secretion remains to be unveiled.

We found that aging enhanced the stimulation of PRL secretion by TRH and PMA, but not by AII and VIP in hormone-replenished AP cells. In response to a maximal dose of TRH ($10^{-7}$ M), PRL secretion was significantly greater in the old than in the young AP cells pretreated with $E_2 + T_3$. This is consistent with the finding that old cycling rats had a greater percentage of AP cells secreting PRL than did young rats under TRH-stimulated conditions

[Larson and Wise, 1991]. On the other hand, based on PRL secretion per lactotrope, these authors reported reduced sensitivity to $10^{-7}$ M TRH in AP cells from old $E_2$-treated rats as compared with young $E_2$-treated rats. However, based on the total PRL releasing capability of the heterogeneous AP cell population, we have demonstrated aging-enhanced responsiveness to TRH. The increased density of TRH receptors observed in the old AP [Donda et al., 1989] is in agreement with our finding. We also observed that the PRL response to $10^{-6}$ M PMA increased during aging in AP cells. This suggests that mechanisms at or after PKC activation may be accentuated in old AP cells. Since PKC is involved in the action of TRH to stimulate PRL secretion [Gershengorn and Osman, 1996; Akita et al., 2000], it appears that the aging-caused changes in the PKC pathway may contribute, in part, to the enhanced TRH effect in old AP cells.

We observed that aging-induced reduction in PRL responses to AII in $E_2$-unprimed AP cells could be abolished by $E_2$ replenishment. In $E_2$-unprimed cells, AII-stimulated PRL secretion was greater in the young than in the old AP cells; however, in $E_2$-primed AP cells, aging did not alter the PRL response to AII. The findings by Janik et al. [1997] that under $E_2$-deprived conditions AII failed to stimulate PRL secretion in aged AP cells but enhanced PRL secretion in a dose-related manner in the young AP cells are in general agreement with our results. $E_2$ is known to increase the PRL response to AII in spite of its ability to decrease AII receptor number in AP cells [Carriere et al., 1986; Pizzi et al., 1992; De Paul et al., 1997; Krishnamurthi et al., 1999]. AII receptor coupling to phospholipase C (PLC) and cross talk to the adenylyl cyclase pathway have been implicated in mediating AII-induced PRL release [Enjalbert et al., 1986; Audinot et al., 1991; Moreau et al., 1994; Lachowicz et al., 1995]. Reduced PRL response to AII in aged AP cells without in vitro $E_2$ priming may reflect the insufficiency of in vivo $E_2$ stimulation to augment intracellular signaling pathways such as PLC and adenylyl cyclase related to the AII action on PRL secretion.

Aging had no effect on VIP-stimulated PRL secretion. VIP at $10^{-9}$, $10^{-7}$, and $10^{-5}$ M caused similar PRL secretion in young and old AP cells with or without $E_2$ priming in vitro. VIP was known to stimulate PRL secretion via the cAMP pathway [Pizzi et al., 1990; Le Pechon-Vallee et al., 2000]. It has also been reported that aging decreased pituitary cAMP and cAMP-binding protein [Arima, 1982]. Our finding that aging did not alter PRL response to VIP suggests that the cAMP pathway and VIP may not play a significant role in the aging-effected high PRL secretion.

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