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Molecular and Cellular Endocrinology 165 (2000) 67–74

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Molecular and Cellular Endocrinology

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# Bromocriptine restores angiotensin II response in pituitary hyperplasia

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Received 17 January 2000; accepted 25 April 2000

**Abstract**

In estrogen-induced pituitary hyperplasia AII-evoked prolactin release is decreased and the octapeptide does not generate a spike elevation in $[Ca^{2+}]_i$ in vitro. We studied whether or not bromocriptine could restore AII response in diethylstilbestrol treated rats. Co-administration of bromocriptine resulted in involution of pituitary size and lowering of serum prolactin. In vitro, prolactin release per cell was reduced in the hyperplastic group, and levels were not significantly increased by in vivo bromocriptine treatment. Immunocytochemical analysis revealed that hyperplastic pituitaries contained fewer prolactin granules than control pituitaries, and that bromocriptine, did not increase prolactin storage. Nevertheless, in this group, prolactin response to AII increased, and AII evoked a consistent spike in $[Ca^{2+}]_i$, albeit lower than in the control group. Such spike was abolished by thapsigargin, and not by removal of extracellular calcium or by $K^+$, indicating that it was mainly dependent on intracellular calcium stores, as in normal cells. We conclude that bromocriptine treatment partially restores AII response in the hyperplastic pituitary. 

*Keywords:* Pituitary; Estrogen; Angiotensin; Bromocriptine; Calcium; Prolactin; Rat

## 1. Introduction

Angiotensin (AII) releases prolactin through a calcium dependent process. In the rat anterior pituitary, AII AT1b receptor stimulation is coupled to phospholipase C mediated hydrolysis of membrane phosphoinositides leading to the formation of diacylglycerol and inositolphosphates, and subsequently to the activation of protein kinase C and to an increase in intracellular calcium levels (Canonico and MacLeod, 1986; Malarkey et al., 1987).

We have recently described that in pituitary cells obtained from in vivo estrogen induced pituitary hyperplasia, the pattern of $Ca^{2+}$ mobilization by AII was altered (Diaz-Torga et al., 1998). AII did not evoke a clear spike elevation of $[Ca^{2+}]_i$ even at high doses ($10^{-5}$ and $10^{-6}$ M). In correlation, the prolactin release in response to different concentrations of AII was lower in this group, even though AII produced a plateau rise in $[Ca^{2+}]_i$ levels. We also demonstrated that in control cells, AII induced a spike response in $[Ca^{2+}]_i$ resulted from mobilization of internal stores by inositol triphosphate, while in hyperplastic cells the principal component of the response was a plateau phase in $[Ca^{2+}]_i$ deriving mainly from $Ca^{2+}$ influx through plasma membrane channels (Iglesias et al., 1999).

It has been shown that bromocriptine treatment reduces pituitary hypertrophy both in humans and in rodents and that it lowers serum prolactin (Thorner et al., 1980; Vrontakis et al., 1987; Bevan et al., 1992). Therefore, in the present set of experiments we evaluated if in vivo bromocriptine treatment in rats with lactotrope hyperplasia induced by chronic estrogen administration, could revert the alteration in AII induced prolactin release and $[Ca^{2+}]_i$ mobilization found in this experimental model.

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0303-7207/00/$ - see front matter 
PII: S0303-7207(00)00270-7

## 2. Materials and methods

### 2.1. Animals

Female 60-days-old Sprague–Dawley rats were housed in an air-conditioned room with lights on at 07.00 and off at 19.00 h. They had free access to laboratory chow and tap water. Rats were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Pituitary hyperplasia (Hyperpl.) was developed by subcutaneous implantation of 20 mg of diethylstilbestrol (DES, Sigma, MO); rats were killed after 8 weeks of treatment. In a group of rats, after six weeks of DES treatment, bromocriptine treatment was started by pellet implantation of 24 mg of the drug (Hyperpl. + Bromo.). Two weeks later (i.e. 8 weeks after DES implantation) rats were killed, trunk blood was collected, and pituitaries were removed for cell dispersion or immunocytochemical studies. Rats in diestrus were used as the control group (Control).

Unless specified, all drugs were purchased at Sigma (MO).

### 2.2. Cell dispersion

Control, Hyperpl. and Hyperpl. + Bromo. pituitaries were removed on ice, separated from the neurointermediate lobe, weighed and placed in chambers containing freshly prepared Krebs–Ringer bicarbonate buffer (KRBGA) without $Ca^{2+}$ or $Mg^{2+}$. Buffer contained 14 mM glucose, 1% bovine seroalbumin, 2% MEM aminoacids (Gibco, Buenos Aires), 1% MEM vitamins, and 0.0025% Phenol Red, and was previously gassed during 15 min with 95% $O_2$ and 5% $CO_2$, and adjusted to pH 7.35–7.40. Buffer was filtered through a membrane (Nalgene) whose pore diameter was 0.45 μm. Hypophyses were washed three times with buffer KRBGA and then cut in 1 mm pieces. Obtained fragments were washed and incubated in the same buffer containing 0.5% trypsin for 30 min at 37°C, 95% $O_2$ and 5% $CO_2$. They were treated for two additional min with DNase I (50 μl of a 1.2 mg/ml solution), and digestion was ended adding 0.2% of newborn calf serum (Gibco). Fragments were dispersed in individual cells by gentle trituration through siliconized Pasteur pipettes. Resulting suspension was filtered through a nylon gauze (160 μm), and centrifuged for 10 min at $120 \times g$. Before centrifugation an aliquot of cellular suspension was taken in order to quantify hypophyseal cell yield, using a Neubauer chamber. Viability of cells determined by Trypan Blue was always greater than 90%. Cells were freshly used for intracellular $Ca^{2+}$ measurements, or plated in sterile cell culture plates.

### 2.3. Cell culture

Cell pellets from the three experimental groups of rats were resuspended in a Dulbecco's modified Eagle medium, supplemented with 10% horse serum, 2.5% fetal calf serum, 1% MEM non-essential aminoacids, 25000 U/l of micostatin and 25 ng/l gentamicin. Cells were plated in sterile tissue culture plates (Corning, Cluster 96; 60 000 cells per well) and incubated with 250 μl Dulbecco Medium (Gibco) in a metabolic incubator at 37°C with 5% $CO_2$ and 95% $O_2$. After long term incubation (96 h), cells were washed twice with Dulbecco's Modified Eagle Medium (with addition of F-12 Nutrient Mixture (Gibco), 2.2 g/l $CO_3HNa$, and 0.1% BSA) to remove all traces of serum. Experimental incubations were performed in 250 μl Dulbecco's Modified Eagle Medium alone (controls) or with $1 \times 10^{-8}$ AII in quadruplicate. For analysis of prolactin secretion, samples were taken at 30 min of incubation period. They were subsequently stored at −20°C until analyzed by radioimmunoassay after appropriate dilution with 0.01 M phosphate-buffered saline with 1% egg albumin. Experiments were repeated at least five times. Time and concentrations were chosen according to our previous experience (Becu-Villalobos et al., 1994).

### 2.4. Radioimmunoassays

Prolactin was measured by RIA using kits provided by the NIDDK. Results are expressed in terms of prolactin PRL $RP_3$. Intra and inter-assay coefficients of variation were 7.2 and 12.8%, respectively.

### 2.5. Immunocytochemistry for prolactin

PRL immunostaining was performed with an antibody generously provided by Dr A.F. Parlow (National Hormone and Pituitary Program, NIH, Bethesda, MD, USA). Pituitaries obtained after decapitation were embedded following routine protocols, as previously described (Piroli et al., 1998). Paraffin sections were cut in a microtome every 4 μm and mounted onto glass slides. Sections were rehydrated, washed in PBS and treated with 0.3% $H_2O_2$ in methanol for 20 min at room temperature to block endogenous peroxidase. The slides were preincubated in 10% normal goat serum at 37°C for 10 min, and a 1:1000 solution containing the first antibody was then added to the sections. After incubating overnight at 4°C, slides were washed with PBS and incubated at room temperature for 1 h with a biotin-labeled second antibody against rabbit IgG, and then with a preformed ABC complex for 30 min (Vector Labs, Burlingame, CA, USA). Subsequently, slides were immersed in a 0.05% 3,3′-diaminobenzidine solution in 0.1 M Tris buffer, pH 7.2 containing 0.01% $H_2O_2$. After 5 min at room temperature, slides were

removed, the reaction was stopped by immersion in PBS, and sections were counterstained with hematoxylin, dehydrated and coverslipped with Permount. Immunoreactive cells were visualized with the aid of an Olympus microscope equipped with a VT-C33ON video camera at a magnification of 400X. The intensity of PRL immunoreactivity was quantified by computerized image analysis (Optimas, Bioscan), as already described (Ferrini et al., 1995). Four animals were used for each experimental group, and a minimum of 4–5 pituitary sections obtained at different levels of each gland were used for quantitation. Results were pooled for the individual animals.

### 2.6. Intracellular $Ca^{2+}$ measurements

Fura-2/AM (tetracetoxymethylesther-Fura 2) was used as a fluorescent indicator. The pellet of adenohypophyseal cells of each experimental group was redispersed and incubated in a buffered saline solution (BSS: 140 mM NaCl, 3.9 mM KCl, 0.7 mM $KH_2PO_4$, 0.5 mM $Na_2HPO_4.12H_2O$, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 20 mM Hepes, pH 7.5) in the presence of FURA-2/AM 1.5 μM, 10 mM glucose, 0.1% BSA. Cells were incubated for 30 min at 37°C in an atmosphere of 5% $CO_2$, time during which FURA-2 is trapped intracellularly by esterase cleavage. Cells were then washed twice in BSS without FURA-2/AM, and brought to a density of $1.7–2 \times 10^6$ cells/ml BSS. Fluorescence was measured in a spectrofluorometer (Jasco Corporation, Tokyo) provided with the accessory CA-261 to measure $Ca^{2+}$ with continuous stirring, thermostat adjusted to 37°C, and injection chamber. Intracellular $Ca^{2+}$ levels were registered every s, by exposure to alternating 340 and 380 nm light beams, and the intensity of light emission at 505 nm was measured. In this way light intensities and their ratio (F340/F380) were followed. Drugs were injected (5 μl) into the chamber as a 100-fold concentrated solution without interruption of recording. The preparation was calibrated determining maximal fluorescence induced by 0.1% Triton X-100 ($F_{max}$) and minimal fluorescence ($F_{min}$) in the presence of 6 mM EGTA (pH adjusted to over 8.3). $[Ca^{2+}]_i$ was calculated according to Grynkiewicz et al. (1985). Basal values were considered as those measured during 20 s before the addition of AII. Values were corrected for dye leakage as described in (Grynkiewicz et al., 1985; Gobbe and Herchuelz, 1989), and for auto fluorescence using unlabeled cells. Both dye leakage and autofluorescence were minimal.

Experiment 1: Responses to 10 nM AII in buffer with or without calcium were compared. In this second case, after incubation with FURA-2/AM, cells were washed and resuspended in BSS without $CaCl_2$ and one min before the experiment 60 μM EGTA was added. These cells did not respond to $K^+$ confirming that no extracellular $Ca^{2+}$ was present (data not shown).

Experiment 2: BSS or 25 mM KCl was injected in the chamber at min 1 and 10 nM AII was applied 2 min thereafter.

Experiment 3: Cell suspensions were incubated in 2 μM thapsigargin for 23 min before AII addition, in order to allow complete inhibition of endoplasmic reticulum ATPases and calcium depletion from this pool (Villalobos and García-Sancho, 1995). This was verified by the inability of 0.1 μM TRH of increasing $[Ca^{2+}]_i$ 23 min after thapsigargin treatment (data not shown).

Where spike and plateau increments are reported, these were defined as the increase over basal levels achieved 12 s after stimulation with AII (spike), or the average increase between 18 and 36 s after AII (plateau).

Time schedules and concentrations of drugs were chosen according to previous experiments (Duvilanski et al., 1998).

### 2.7. Statistical analyses

Results are expressed as mean ± S.E. Peak values or plateau levels at determined time periods were analysed by two-way analysis of variance for repeated measures. Individual means were then compared by Duncan's test. Hormone secretion in vitro and in vivo, and pituitary weights were analysed by one-way analysis of variance. $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Serum prolactin and pituitary weight

Pituitary weight increased 3.0 fold after 6 weeks of estrogen treatment. When bromocriptine was administered for the last 2 weeks, pituitary weight increase (1.6-fold) was significantly lower than in the Hyperpl. group. Nevertheless, it remained higher than in the Control group (Fig. 1). Serum prolactin levels varied accordingly, in the Hyperpl. group, there was a 47.3-fold increase in comparison to the Control group, while in the Hyperpl + Bromo. group, there was only a 6.7-fold increase.

### 3.2. Basal prolactin release and storage

Primary culture studies revealed that basal levels of prolactin release per cell were reduced in the Hyperpl. and in the Hyperpl. + Bromo. group in comparison to the Control group (Control 377 ± 38, Hyperpl. 228 ± 18 and Hyperpl. + Bromo. 250 ± 13 ng/ml, $P < 0.05$ control vs. Hyperpl. and Hyperpl. + Bromo., $n = 4$).

Immunocytochemical studies of the pituitaries revealed that even though the Hyperpl. and Hyperpl. +

Bromo. groups contained hypertrophic cells, prolactin immunoreactivity was greatly reduced in comparison to the control group (Fig. 2a–c, Fig. 3).

### 3.3. AII- induced prolactin release

A 30 min exposure to 10 nM AII significantly increased prolactin release in the Control group, there was a net increment of $101.3 \pm 8.3$ ng/ml which represented an average percent increase of $29.6 \pm 4.1$ (Fig. 4). As previously described AII only scarcely increased prolactin levels in the Hyperpl. group, there was a net, not significant, increment of $42.3 \pm 4.3$ ng/ml, which represented a percent increase of $13.7 \pm 3.7$ ($P < 0.05$ for both values vs. Control). In the Hyperpl. + Bromo. group, AII evoked a significant increase of $62.2 \pm 7.3$ ng/ml ($P < 0.05$ vs. Control and Hyperpl.), which represented a percent increase of $25.1 \pm 1.5$ (NS vs. Control group, $P < 0.05$ vs. Hyperpl. group).

### 3.4. AII-induced intracellular $Ca^{2+}$ mobilization

In pituitary cells from female diestrous rats 10 nM AII induced a spike response of $[Ca^{2+}]_i$ (Fig. 5). It consisted of a 2–6 s delay, a peak increase of 45.8% (rise from $192.5 \pm 3.1$ nM to a peak of $280.7 \pm 6.3$ nM), after $12 \pm 2$ s and a subsequent decay to resting levels after $50 \pm 3$ s. From min 1 to 2 after AII stimulus, $[Ca^{2+}]_i$ remained slightly below resting levels ($-1.8\%$).

In Hyperpl. cells the spike phase was practically absent (Fig. 5) and there was a plateau increase of 9% over basal levels. 10 nM AII increased $[Ca^{2+}]_i$ from $201.5 \pm 9.0$ nM, to a plateau concentration of $219.6 \pm 7.4$ nM after $21 \pm 2$ s, and levels remained elevated for at least 3 min.

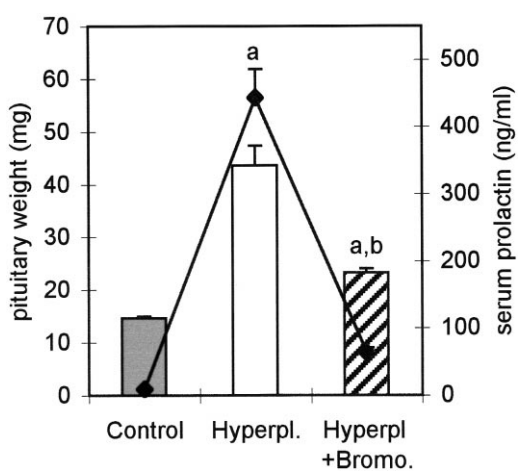


Fig. 1. Serum prolactin levels (ng/ml, line) and pituitary weight (mg, bars) in control, diethylestilbestrol (Hyperpl.) and diethylestilbestrol + bromocriptine (Hyperpl. + Bromo.) treated rats. (a) $P < 0.05$ vs. control; (b) $P < 0.05$ vs. Hyperpl.

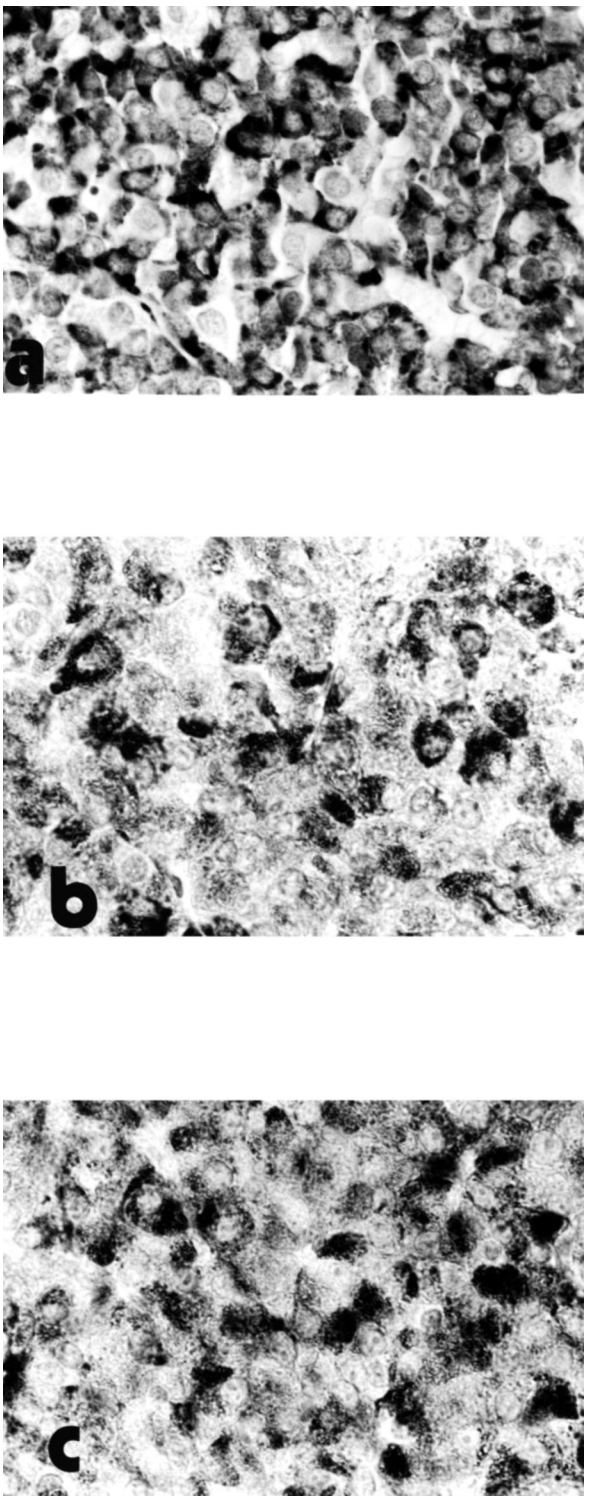


Fig. 2. Immunocytochemistry for prolactin in anterior pituitaries from control (panel a), diethylstilbestrol (panel b) and diethylstilbestrol + bromocriptine (panel c) treated rats.

In cells from estrogen induced pituitary hyperplasia with bromocriptine treatment, there was a significant spike increase (which was still lower than in the control group). It consisted of a 2–6 s delay, a rise from $194.5 \pm 3.2$ nM to a peak of $223.3 \pm 4.6$ nM (14.8%)

after $15 \pm 2$ s, and a subsequent decay to a plateau level.

### 3.5. Participation of plasma membrane $Ca^{2+}$ channels mediating AII action in cells from estrogen induced hyperplasia attenuated by bromocriptine treatment

In order to assess the relative contribution of $Ca^{2+}$ influx in AII action, we tested the effect of 10 nM AII on $[Ca^{2+}]_i$ in the absence of extracellular calcium, or in the presence of a depolarizing agent (25 mM $K^+$).

Removal of extracellular $Ca^{2+}$ consistently reduced basal $[Ca^{2+}]_i$ in the three groups (average decrease $27.3 \pm 9.8\%$). Spike phase response to 10 nM AII was unaltered in control and in the Hyperpl. + Bromo. group (Control with and without extracellular calcium: $40.2 \pm 3.6$ and $38.8 \pm 5.6\%$, NS; Hyperpl. + Bromo.: $17.6 \pm 3.2\%$ and $15.1 \pm 0.1\%$, NS) (Fig. 6). Furthermore, plateau response in the Hyperpl. + Bromo group was inhibited, and plateau increase in the Hyperpl. group was abolished.

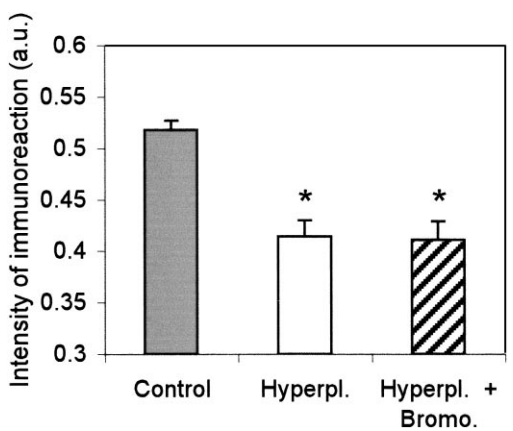


Fig. 3. Quantification of prolactin content by immunocytochemistry in the three experimental groups. a.u., arbitrary units. * $P < 0.05$ vs. control.

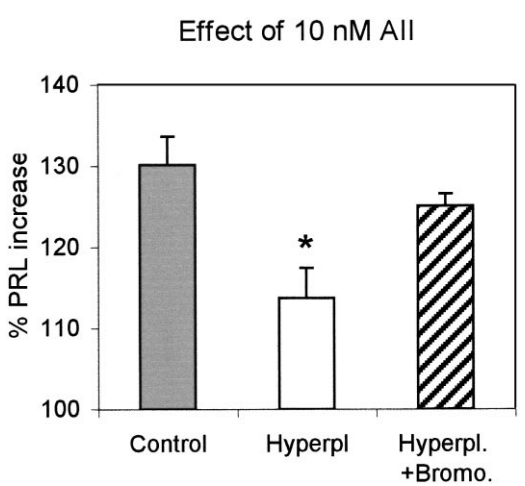


Fig. 4. Percent prolactin release induced by a 30 min stimulus of 10 nM AII in cultured anterior pituitary cells from control, diethylstilbestrol (Hyperpl.) and diethylstilbestrol + bromocriptine (Hyperpl. + Bromo.) treated rats. * $P < 0.05$ vs. control group. $n = 4$ experiments.

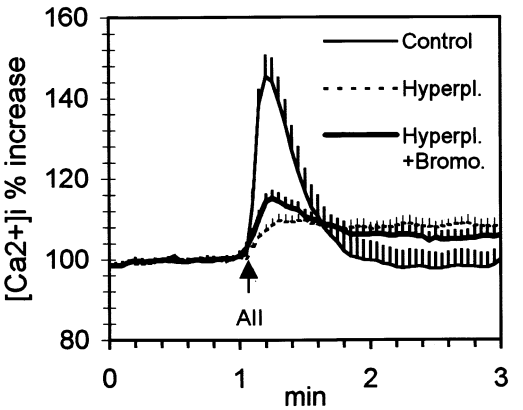


Fig. 5. Effect of 10 nM AII on percent variation of $[Ca^{2+}]_i$ in dispersed anterior pituitary cells from female rats in diestrus (control), from estrogen induced pituitary hyperplasia (Hyperpl.) and from estrogen induced hyperplasia + bromocriptine treatment (Hyperl. + Bromo.). AII was applied at min 1 (arrow). $n = 6$ experiments for each group ($n$ always indicates number of experiments on different days, each performed in replicate from a batch of cells derived from at least 3 rats). For this and the following figures, lines represent average for each time point, thin lines on top or below, 1 S.E. Results are expressed as % increase of $[Ca^{2+}]_i$ over basal levels (basal levels were considered as the average of $[Ca^{2+}]_i$ 20 s before the AII stimulus).

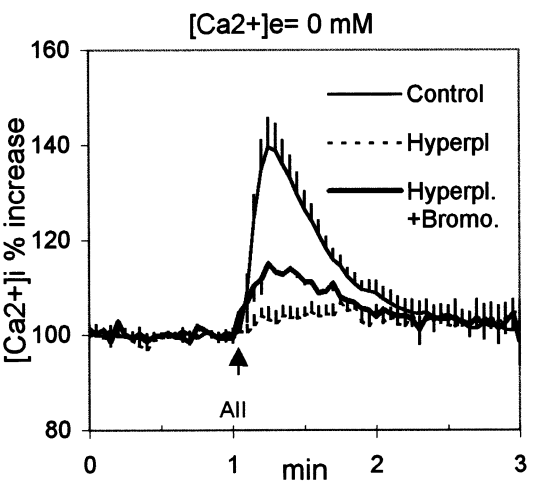


Fig. 6. Effect of 10 nM AII on $[Ca^{2+}]_i$ in BSS buffer without $Ca^{2+}$ and with 60 μM EGTA in dispersed anterior pituitary cells from female rats in diestrus (control), from estrogen induced pituitary hyperplasia (Hyperpl.) and from estrogen induced hyperplasia + bromocriptine treatment (Hyperpl. + Bromo.). $n = 3$ for each group.

High extracellular $K^+$ concentration depolarizes the cells and stimulates $Ca^{2+}$ influx through voltage sensitive calcium channels (VSCC). When extracellular $K^+$ was increased to 25 mM, there was an immediate, significant and transient increase in $[Ca^{2+}]_i$ in the three experimental groups, which reached a peak at $14 \pm 2$ sec. The $[Ca^{2+}]_i$ subsequently dropped, initially rapidly,

then gradually and remained at a plateau level. Spike $Ca^{2+}$ response evoked by 10 nM AII was unaltered by $K^+$ pretreatment in control cells, and was slightly decreased in the Hyperpl. + Bromo. group (Fig. 7). Increments achieved by AII in the presence and absence of $K^+$ were of $7.8 \pm 1.8$ and $15.4 \pm 2.6\%$, respectively ($P < 0.05$) in this last group, and $31.2 \pm 2.4$ and $38.7 \pm 6.9\%$ (NS) in control cells. These results suggest that an important component of the spike rise in $[Ca^{2+}]_i$ induced by AII in the Hyperpl. + Bromo. was independent of VSCC activation. On the other hand, $K^+$ pretreatment abolished plateau-increase of $[Ca^{2+}]_i$ in Hyperpl. cells, and the plateau component of response in the Hyperpl. + Bromo group.

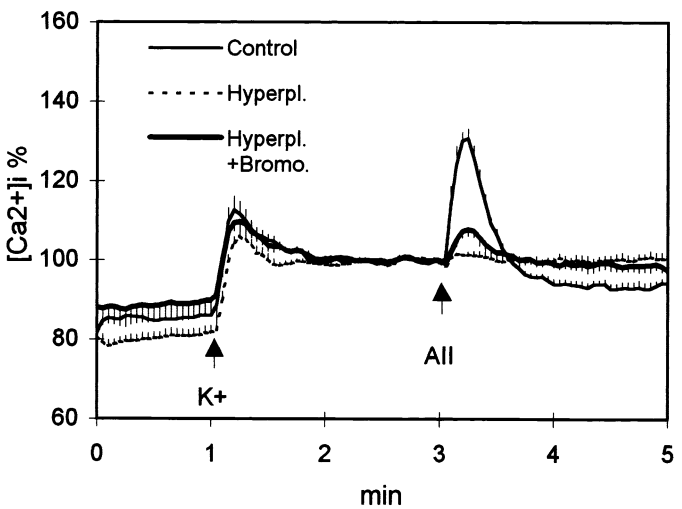


Fig. 7. Effect of administration of 25 mM $K^+$ (min 1) on percent variation of $[Ca^{2+}]_i$ induced by 10 nM AII (min 3) in dispersed anterior pituitary cells from female rats in diestrus (control), from estrogen induced pituitary hyperplasia (Hyperpl.) and from estrogen induced hyperplasia + bromocriptine treatment (Hyperpl. + Bromo.). $n = 4$ for each group.

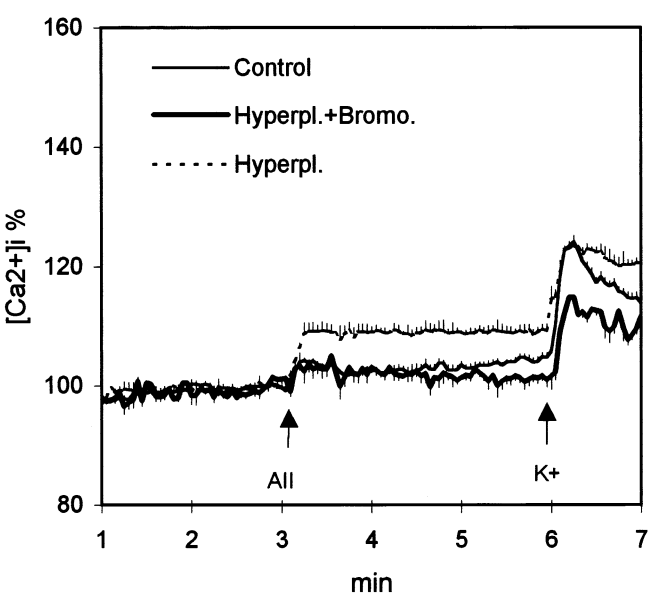


Fig. 8. Effect of depleting intracellular calcium stores with 2 μM thapsigargin (23 min of pretreatment) on AII induced $Ca^{2+}$ mobilization in dispersed anterior pituitary cells from female rats in diestrus (control), from estrogen induced pituitary hyperplasia (Hyperpl.) and from estrogen induced hyperplasia + bromocriptine treatment (Hyperpl. + Bromo.). 10 nM AII at min 3, and 25 mM $K^+$ at min 6. $n = 4$ for each group.

### *3.6. Participation of thapsigargin sensitive $Ca^{2+}$ pools mediating AII action in cells from estrogen induced hyperplasia attenuated by bromocriptine treatment*

The relative $Ca^{2+}$ content of the intracellular $Ca^{2+}$ pools was estimated by mobilizing reticular stored $Ca^{2+}$ to the cytoplasm. To that end we used thapsigargin, the specific inhibitor of the endoplasmic reticulum $Ca^{2+}$ pumps. Two micromolar thapsigargin elicited a peak in $[Ca^{2+}]_i$ followed by plateau increase (data not shown). The response was similar in Control, Hyperpl. and Hyperpl. + Bromo. cells, absolute spike increments were $53.9 \pm 8.3$, $59.9 \pm 6.0$ and $46.3 \pm 5.3$ nM, respectively (NS).

Pretreatment with 2 μM thapsigargin prevented the AII-induced $[Ca^{2+}]_i$ spike increase in control cells and in Hyperpl. + Bromo. cells (Fig. 8). On the other hand plateau phase elicited in the Hyperpl. group was not modified.

## 4. Discussion

The semi synthetic ergot bromocriptine is a potent inhibitor of prolactin secretion in vivo and in vitro. It binds to anterior pituitary dopamine receptors of the D2 subtype with high affinity and suppresses prolactin secretion and lactotroph proliferation. Besides, it is effective in decreasing estrogen-induced pituitary enlargements in rats, and the size of prolactin secreting tumors in humans (Vrontakis et al., 1987; Thorner et al., 1980; Bevan et al., 1992). It also abolishes the concomitant arteriogenesis that occurs in the estrogen treated anterior pituitary (Elias and Weiner, 1987).

In previous works, we described that in pituitary hyperplasia due to in vivo chronic estrogen treatment, AII regulation of prolactin secretion was altered. The prolactin releasing effect of AII was greatly reduced, and this effect was specific as the response to TRH was increased. Concomitantly $[Ca^{2+}]_i$ mobilization in response to graded concentrations of AII presented a different profile (Diaz-Torga et al., 1998) in hyperplastic cells. While in cells from rats in diestrus AII induced a biphasic increase in $[Ca^{2+}]_i$, characterized by an initial spike and a posterior plateau phase, in cells from pituitary hyperplasia the spike phase was lost, and only a plateau increase could be observed. We also determined that estrogen treatment lowered AII receptors in

the pituitary (Seltzer et al., 1992). Finally, we described that there were differences in the subcellular pathways and sources of $Ca^{2+}$ involved in the increase in $[Ca^{2+}]_i$ evoked in normal and hyperplastic cells (Iglesias et al., 1999). Spike increase in $[Ca^{2+}]_i$ in normal cells could be abolished by thapsigargin pretreatment, indicating that the early effect of AII was dependent on $Ca^{2+}$ reticular stores of the cells. In contrast, in hyperplastic cells the plateau response was not inhibited by thapsigargin, but was greatly reduced or abolished by manipulations of plasma membrane calcium channels. Nifedipine, verapamil (blockers of L-type VSCC) and removal of extracellular calcium led to a significant reduction of the plateau response, while a depolarizing concentration of $K^+$ abolished the response in these hyperplastic cells.

In the present experiments, we wished to determine whether or not a pharmacological reduction of pituitary enlargement and of serum prolactin concentration in estrogen treated rats could restore AII induced prolactin release and normalize $Ca^{2+}$ mobilization evoked by the octapeptide. To that end, estrogen treated rats were implanted with a sc pellet of bromocriptine 2 weeks before sacrifice. As expected, coadministration of bromocriptine with estrogen resulted in an involution of pituitary size and lowering of serum prolactin, indicating the overriding influence of dopamine in spite of a continued estrogenic stimulus.

When cells were plated for in vitro studies, it was observed that prolactin release per cell was reduced in the hyperplastic group, and in vivo bromocriptine did not significantly increase those low levels. Reduced levels could not be the result of lower percentage of lactotrophs, as it has been repeatedly shown that estrogen elevates the percentage of lactotrophs in the pituitary (Banerjee et al., 1994). It is probable that due to the high exocytotic rate of hyperplastic lactotrophs, these become degranulated. In fact, our immunocytochemical studies showed that Hyperpl. pituitaries contained less immunoreactive prolactin. To this respect, it has been described that after estrogen implantation for 6 weeks, lactotrophs display evidence of high secretory activity, and often relatively few small secretory granules (Drewett et al., 1993). It was also shown that after 4 or 7 weeks of estrogen treatment, most of the coarse granulated, darkly stained cells seen in control pituitary cells become degranulated and hypertrophic (Casanueva et al., 1982). As a result, pituitary prolactin concentration (μg/mg tissue) significantly decreases from 2 to 8 weeks of estradiol treatment, but serum prolactin levels rise due to marked pituitary enlargement (Lawson and Parker, 1991).

Treatment of prolactin cells with a dopaminergic agent induced increased secretory granules in the cytoplasm of pituitary cells (Eguchi et al., 1995; Matsuno et al., 1995), but in our present experimental model the effect of bromocriptine was not significant in relation to prolactin content and basal release.

Nevertheless, AII induced prolactin release was greater in the Hyperpl. + Bromo group in comparison with the Hyperpl. group, even though bromocriptine treatment did not completely restore the response. When we analyzed intracellular $Ca^{2+}$ mobilization induced by AII, we found that in contrast to the plateau response in Hyperpl. cells, in the Hyperpl. + Bromo. group AII evoked a consistent early spike in $[Ca^{2+}]_i$, albeit lower than in the control group. We next evaluated calcium channels participating in such response, and found that thapsigargin, which depletes intracellular reticular stores, prevented the spike in $[Ca^{2+}]_i$ induced by AII in a similar way as described in control cells (Iglesias et al., 1999). Besides, removal of extracellular $Ca^{2+}$ did not abolish the spike phase in Hyperpl. + Bromo. cells or in control cells. These data suggest that in both groups AII mobilized an intracellular $Ca^{2+}$ store. In contrast, thapsigargin did not modify the $Ca^{2+}$ response in Hyperpl. cells and removal of extracellular $Ca^{2+}$ prevented the response. Finally, depolarization of VSCC by 25 mM $K^+$ reduced but did not abolish spike increase in Hyperpl. + Bromo. cells. It is therefore probable that even though the main component of the attenuated spike increase in $[Ca^{2+}]_i$ induced by AII depends on mobilization of intracellular calcium stores, calcium influx through VSCC might also contribute. To this respect, it was evident that in the Hyperpl. + Bromo. cells the plateau $[Ca^{2+}]_i$ increase which followed the spike increase, was lowered by previous depolarization with $K^+$ or by incubating cells in nominally $Ca^{2+}$ free buffer. This demonstrates that in the presence of AII, cells from estrogen induced hyperplasia attenuated by in vivo bromocriptine treatment present a spike $[Ca^{2+}]_i$ increase principally dependent on intracellular stores as in the case of control cells, and a later plateau phase, dependent on calcium influx as in hyperplastic cells. This partial restoration of $Ca^{2+}$ mobilization by AII is in agreement with results of prolactin secretion, in which there was also only a partial restoration of the effect of AII.

Interaction of the dopaminergic system and AII has been documented in other tissues. For example, chronic administration of an estrogenic agent attenuated the drinking response of rats to AII, and bromocriptine reverted the AII induced water intake (Fregly, 1987). On the other hand, dopamine and AII receptors have been reported to interact negatively: After treatment with bromocriptine, AII binding sites as well as AII induced response were reduced in renal proximal tubules (Hussain et al., 1998). Finally, activation of dopaminergic receptors resulted in a decrease of the renin-AII system in hypertensive patients (Luchsinger et al., 1992). In the present experiments we show that bromocriptine treatment partially restores AII response in the hyperplastic pituitary, and that it antagonizes the effect of estrogen. As the response to AII was increased

by bromocriptine, it is probable that the interaction of the dopamine and renin-AII systems is tissue specific.

## Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina; Universidad de Buenos Aires and Agencia Nacional de Promoción Científica y Tecnológica, Argentina. We thank NIDDK's National Hormone and Pituitary Program and Dr A.F. Parlow for the RIA kits and antiserum for immunocytochemical studies.

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