IN MAMMALS, THE RENIN-ANGIOTENSIN SYSTEM (RAS) IS A COMPLEX REGULATORY SYSTEM GENERATING OCTAPEPTIDE ANGIOTENSIN II (ANG II), THE BIOLOGICALLY ACTIVE PROPEPTIDE. SIGNALS FOR ANG II ACTIVATION, INCLUDING DECREASES IN GLomerular blood flow and in plasma sodium concentration, LEAD TO RENIN RELEASE FROM THE JUXTAGLOMERULAR APPARATUS OF THE KIDNEY INTO THE CIRCULATION, WHERE IT ACTIVATES ANG II PRODUCTION IN VITRO BY ACE AND ANG II. AM J PHYSIOL 273 (REGULATORY INTEGRATIVE COMP PHYSIOL 42): R2089–R2096, 1997.—Our aim was to study the role of angiotensin-converting enzyme (ACE) and angiotensin II (ANG II) in ovarian steroidogenesis and prostaglandin production in amphibian. Hormonal effects of ACE, ACE inhibitors, synthetic bullfrog angiotensin I (ANG I), and [Val]ANG II were compared on frog ovaries of postreproductive and prereproductive periods. Very high ACE activity was found in ovary of water frog (Rana esculenta) compared with other frog tissues, and this activity was inhibited by the typical ACE inhibitors, captopril and lisinopril. Frog ovary tissue in postreproductive and prereproductive periods was incubated in vitro in the presence of ACE (2.5 µU/ml), captopril (0.1 mM), lisinopril (0.1 mM), [Val]ANG II (1 µM), and synthetic bullfrog ANG I (1 µM). Production of 17β-estradiol, progesterone, androgens, and prostaglandins E₂ and F₂α was determined. The data showed a modulation of 17β-estradiol, progesterone, and androgens, and prostaglandins E₂ and F₂α production by ovary ACE; on the other hand, [Val]ANG II modulated the production of progesterone and prostaglandin F₂α, whereas androgen production was not influenced. The present in vitro studies suggest the existence of two pathways independently regulated by ACE and ANG II modulating ovarian steroidogenesis and prostaglandin production. 

17β-estradiol; progesterone; androgens; prostaglandin E₂; prostaglandin F₂α.

The authors, in fact, present evidence showing that the ovarian RAS can regulate normal ovarian function by paracrine and intracrine mechanisms. Furthermore, Daud et al. (9) indicated the presence of high levels of biologically active ACE in the surface of ovary granulosa cell. In addition, they demonstrated a lack of effect of ACE on follicular maturation and ovulation, using short- and long-term (6 and 14 days) infusions of the ACE inhibitor captopril on ovulation induced in immature rats by the intraperitoneal injection of pregnant mare’s serum gonadotrophin (PMSG) and human choriionic gonadotrophin (hCG). Peterson et al. (24) demonstrated that ACE inhibitors have no effect on ovulation and ovarian steroidogenesis in the perfused rat ovary and that ACE inhibition via captopril and teprotide does not result in ANG II antagonist effects; moreover, ANG II was shown to be an important mediator in the mechanism of ovulation. Pellicer et al. (22) showed the direct involvement of ANG II in the ovulation of immature rats in which follicle development and ovulation had been induced with PMSG and hCG. Yoshimura et al. (37) demonstrated that ANG II stimulated the production of both estradiol and prostaglandins by perfused rabbit ovaries in the absence of gonadotrophin. The addition of an ANG II receptor antagonist,
saralasin, to the perfusate blocked the hCG-stimulated production of estradiol and prostaglandins in a dose-dependent manner. In rats, ANG II also stimulates the leutinizing hormone (LH) preovulatory peak in the hypothalamus (30).

The RAS is also considered to be important in amphibians such as frogs, in which the same components of the system have been found (34). The presence of renin activity in the plasma and kidney of various frog species (28) (such as Rana catesbeiana) and the generation of [Val5,Asn9]ANG I by the incubation of bullfrog plasma with a renal extract of bullfrog (16) have been reported. Bullfrog ANG I is thought to be physiologically inactive, similar to mammalian ANG I, and it becomes active when converted into ANG II by some converting factors (16). The presence of an enzyme that corresponds to mammalian ACE has been proved in bullfrog, showing a large amount of ACE bound to membrane in the kidney (35). Our work has demonstrated the presence of ACE in serum of newt (Triturus carnifex) and frog (Rana esculenta) (19).

The objective of this paper was to study the role of ACE and ANG II on ovarian steroidogenesis and prostaglandin production in amphibian. We followed the production of estradiol, progesterone, androgens, and prostaglandins (PG) E2 and F2α in vitro incubation of ovarian tissue of the water frog (R. esculenta) in postreproductive and prereproductive periods. The choice of the frog ovary is suggested for two reasons: first, the lack of literature about the influence of ovarian ACE in amphibian steroidogenesis and, second, the well-known characterization of the ovarian steroids and prostaglandin production during the different periods of the amphibian annual breeding cycle (15). By studying the paracrine action of angiotensin on the in vitro ovary, some of the contradictory role of ANG I, ACE, and ANG II found in mammals may be better understood.

MATERIALS AND METHODS

Chemicals

- N-[3-(2-furyl)acryloyl]-L-phenylalanine-glycyl-glycine (FAPGG), N-[3-(2-furyl)acyrloyl]-L-phenylalanine (FAP), [Val5]ANG II (Asp-Arg-Val-Tyr-Val-His-Pro-Phe), bullfrog ANG I (Asp-Arg-Val-Tyr-Val-His-Pro-Phe-Asn-Leu), ACE (rabbit lung), captopril, lisinopril, Dulbecco's modified Eagle's medium (DMEM), penicillin G, streptomycin, progesterone, testosterone, 17β-estradiol, PGF2α, and PGE2 were purchased from Sigma (St. Louis, MO).

- Falcons (vaccine for bovine serum albumin) was from Behringer Mannheim, and trypsin inhibitor (bovine lung) was from Serva Feinbiochemica. High-pressure liquid chromatography (HPLC) column was Supelcosil LC-318 from Supelco (Bellevente, PA). Multiwell tissue culture plates were from Becton Dickinson (Lincoln Park, NJ). Progesterone, androgens, 17β-estradiol, and PGF2α antiserum were provided by Dr. G. F. Bolelli [Consiglio Nazionale delle Ricerche (CNR)-Institute of Normal and Pathologic Cytomorphology, University of Bologna, Bologna, Italy] and Dr. F. Franceschetti (CNR-Physiopathology of Reproduction Service, University of Bologna, Bologna, Italy), and the PGE2 antiserum was purchased from Cayman Chemical (Ann Arbor, MI).

- [1,2,6,7-3H]progesterone, [1,2,6,7-3H]testosterone, [2,4,6,7-3H]17β-estradiol, [5,6,8,9,11,12,14,15(n)-3H]PGE2, and [5,6,8,11,12,14,15(n)-3H]PGF2α were purchased from Amersham (Buckinghamshire, UK).

Animals

Adult female frogs (average weight, 25 g), R. esculenta, were collected in Umbria, Italy, from a pond (870 m above sea level). This frog population breeds in May (reproductive period), when the temperature increases, and enters a postreproductive period in the summer. Gonad recrudescence is initiated in midsummer and continues into the autumn (recovery period). The animals hibernate during the cold months of winter in ground shelters (hibernation period) to emerge when the temperature increases in the following spring. At the beginning of spring, the frogs return to the pond (prereproductive period).

Preparation of Crude Homogenates, Tissue Membranes, and Ovary Trypsin Extraction

All procedures described below were carried out at 4°C. For each extraction, six adult female frogs, R. esculenta, were killed by decapitation; ovaries, kidneys, and lungs were removed and pooled, and tissues were weighed. Tissues were finely minced with scissors and homogenized in 10 volumes of ice-cold phosphate buffer (50 mM, pH 8.3) with a Braun homogenizer set at the highest speed for 5 min. The homogenate was filtered through cotton gauze and then centrifuged at 1,000 g for 20 min. The supernatant was decanted and used for determination of the content of FAPGG hydrolyzing activity. Tissue membranes were isolated by ultracentrifugation of supernatant at 100,000 g at 4°C for 60 min. The resulting pellet was resuspended in 0.5 or 1 ml of phosphate buffer and assayed for FAPGG hydrolytic activity (27). The extraction was carried out by treating the ovary membrane suspension with 5 mg trypsin/g protein for 1 h at 37°C. The incubation was repeated again, with the addition of fresh trypsin preparation. Trypsin activity was quenched with bovine lung trypsin inhibitor at a 4:1 ratio with trypsin. The solution was centrifuged at 100,000 g for 20 min. The supernatant was assayed for FAPGG hydrolytic activity. Protein content was evaluated by the method of Bradford (5), with bovine serum albumin as standard.

Experimental Protocol

In vitro studies. To study the ovarian steroidogenesis and prostaglandin production, female frogs from the postreproductive and prereproductive periods were captured and killed in the field by decapitation. The ovaries were removed, placed in cold DMEM containing 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 0.1 mg/ml penicillin G, and 0.1 mg/ml streptomycin, and transferred to the laboratory, where they were divided into equal-sized fragments, pooled, and equally distributed over incubation wells (1 g/well). Each incubation set of wells was divided into six experimental groups (each consisting of four wells): 1) medium alone, 2) medium plus 2.5 µM rabbit lung ACE, 3) medium plus 0.1 mM captopril, 4) medium plus 0.1 mM lisinopril, 5) medium plus 0.1 mM captopril plus 2.5 µM rabbit lung ACE, and 6) medium plus 0.1 mM lisinopril plus 2.5 µM rabbit lung ACE. [Val5]ANG II and synthetic bullfrog ANG I, at a final concentration of 1 µM, were added to a second and third incubation set, respectively. In a fourth incubation set 2.5 µM rabbit lung ACE was replaced with 2.5 µM trypsinated frog ovary
ACE. Culture plates were wrapped in aluminum foil and incubated at room temperature. Incubation medium was removed after 6 h and stored at −20°C until hormone assays. Ovarian tissues were homogenized in amphibian saline, and protein contents were determined with the use of a commercial kit (Bio-Rad, Richmond, CA) (5). The control experiment was repeated with incubation media without ovarian tissue.

**Determination of FAPGG hydrolyzing activity.** FAPGG hydrolytic activity was measured as follows. Enzyme solutions (4 µl) from fractions of different steps of purification were incubated at 37°C at different times in the presence of FAPGG in 80 mM borate buffer, pH 8.2, containing 300 mM NaCl in a final volume of 40 µl. The enzymatic reaction was stopped by adding 4 µl of 5% TFA and centrifuged. FAPGG and FAP separations were performed on a Perkin-Elmer high-pressure liquid chromatograph (Series 22) using a 5-µm Supelcosil LC-318 (25 cm × 4.6 mm ID) column, protected with a 5-µm Supelcosil LC-318 guard column (2 cm × 4.6 mm ID). The injection volume of the incubation mixture was 20 µl. Elution was performed with 30% of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) at a constant flow rate of 1 ml/min. Substrate and product(s) were monitored by ultraviolet (UV) absorbance at 300 nm and recorded with a Chromatoarrayer 12 computing integrator (Perkin-Elmer). The rate of FAPGG hydrolysis was computed from the peaks of the FAP product by establishing calcium calibration curves of the peak areas with the use of various amounts of FAP. Enzyme activity is expressed as nanomoles of FAP per milligram of protein or grams of tissue per minute of incubation at 37°C.

**Determination of kinetic parameters.** Specific ACE inhibitors, captopril and lisinopril, were tested at concentrations ranging from 0.1 nM to 10 µM under routine enzyme assay conditions (see above), and inhibitions were expressed as percentages of the reference activity measured in the absence of chemical reagents under the same experimental condition. Half-maximal inhibition (IC50) values were obtained from the inhibition curves. Inhibition assays were run on average two or three times. Results are expressed as means ± SE. Kinetic studies were evaluated on ovary tissue membrane preparations. The concentration of FAPGG was varied from 50 µM to 5 mM, and the concentration of ovary tissue membrane proteins was 1.6 µg/ml. Initial rates were measured for the first 10 min of reaction. Kinetic parameters were evaluated by linear regression analysis using Fig. P (Biosoft, Cambridge, UK).

Hydrolysis of ANG I by homogenate and membrane suspension of ovary. Homogenate (86 µg) or membrane suspension (3.2 µg) of ovary was added to 100 µM synthetic bullfrog ANG I solution in 80 mM borate buffer, pH 8.2, containing 300 mM NaCl in a total volume of 20 µl. The solution was incubated at 37°C for 5 min. TFA was used to acidify to pH 2.0, stopping the incubation, and the solution was injected for reverse-phase HPLC analysis using a 5-µm Supelcosil LC-318 column as described above. The elution was performed with linear gradient from 15 to 35% of 0.1% TFA in water and 0.1% TFA in acetonitrile at a flow rate of 1 ml/min. Eluate absorbance was monitored by UV absorbance at 214 nm. Identification of the ANG II peak was facilitated by adding 1 nmol of [Val5] ANG II to the mixture after incubation and before injection of the sample for reverse-phase HPLC analysis, or by incubating the mixture with 0.1 mM captopril.

Determination of progesterone, androgens, 17β-estradiol, PGF2α, and PGE2. Concentrations of progesterone, androgens, 17β-estradiol, PGF2α, and PGE2 were measured in incubation media by radioimmunoassay as described previously (10, 15). Intra- and interassay coefficients of variation and minimum detectable doses were, respectively, progesterone, 7%, 11%, 12 pg; testosterone, 9%, 14%, 8 pg; 17β-estradiol, 6%, 10%, 11 pg; PGF2α, 8%, 14%, 16 pg; and PGE2, 9%, 13%, 14 pg. Testosterone was not separated from 5α-dihydrotestosterone and, therefore, because the antiserum used is not specific, the data are expressed as androgens.

**Statistical Analysis**

An analysis of variance followed by Duncan’s multiple-range test (29) was used to analyze the data.

**RESULTS**

At first, hydrolyzing activity contents toward FAPGG, a synthetic substrate of ACE (17), were measured in ovary, lung, and kidney of frog (R. esculenta) (Table 1). The reported values show FAPGG hydrolyzing activity of the crude tissue homogenates, expressed as micromoles per minute of FAP product referred to grams of tissue or milligram of proteins in the homogenate. Analysis of data shows the presence of FAPGG hydrolyzing activity in lung and kidney, as proved by Yamaguchi et al. (35) in bullfrog. Interestingly, the highest content of FAPGG hydrolyzing activity was in the ovary, ~10 times the content in lung and kidney tissues.

To prove that ACE is responsible for FAPGG hydrolyzing activity, a partial purification of the enzyme was carried out from frog ovary, lung, and kidney tissues using the procedure of Smiley and Doig (27). The method consists of the purification of ACE bound to the membranes by using ultracentrifugation of homogenate and recovery of the particulate fractions. The analysis of FAPGG hydrolyzing activity present in the pellets is similar to the results obtained by Smiley and Doig and confirms the data obtained from the total homogenates. Due to the high FAPGG hydrolyzing activity present in frog ovary, this result prompted us to characterize this enzyme activity better.

Figure 1 reports the markedly inhibited enzymatic activity of purified membrane suspension in the presence of specific ACE inhibitors (3), captopril and lisinopril, at different concentrations. IC50 values, obtained from the inhibition curves, were 66.19 ± 0.297 nM for captopril and 4.062 ± 0.529 nM for lisinopril, using FAPGG as substrate. The values are very close to those reported in the literature for ACE (6). With the use of a computing program, linear regression analysis of FAPGG hydrolysis by ovary membrane preparation gave a Michaelis-Menten constant of 0.264 ± 0.046 mM.

**Table 1. Contents of FAPGG hydrolyzing activity in ovary, lung, and kidney of frog (Rana esculenta).**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>µmol·min⁻¹·g⁻¹</th>
<th>µmol·min⁻¹·mg⁻¹·protein⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>26.580 ± 0.374</td>
<td>0.2972 ± 0.0042</td>
</tr>
<tr>
<td>Lung</td>
<td>2.764 ± 0.138</td>
<td>0.0376 ± 0.0019</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.047 ± 0.374</td>
<td>0.0228 ± 0.0011</td>
</tr>
</tbody>
</table>

Data are means ± SE of 3 replicate assays from pooled tissues of 6 female frogs (n = 6). FAPGG, N-[3-(2-furyl)acryloyl] L-phenylalananyl-glycyl-glycine.
and a maximum velocity of 626.976 ± 46.171 nmol·min⁻¹·mg protein⁻¹. To prove further the presence of ACE activity on frog ovary membranes, synthetic bullfrog ANG I was incubated at 37°C at different times in the presence of purified ovary membranes. The hydrolytic products were analyzed by reverse-phase HPLC following the procedure described in MATERIALS AND METHODS. ■ Captopril; ▲ lisinopril.

ACE activity in the frog ovary and prompted us to study the physiological function of ACE on steroidogenesis and prostaglandin synthesis in frog ovary.

Frog ovary tissue in postreproductive period was incubated in vitro in the presence of rabbit lung ACE, captopril, lisinopril, [Val⁵]ANG II, and synthetic bullfrog ANG I. The production of 17β-estradiol, progesterone, androgens, PGE₂, and PGF₂α was determined.

Figure 3A shows 17β-estradiol production, expressed as picograms per milligram of protein, by frog ovary incubated in vitro. A 17β-estradiol basal value of 360 ± 21 pg/mg protein was inhibited by adding rabbit lung ACE at the final concentration of 2.5 μU/ml. The same results were obtained with 1.5 μU/ml. Treatment with specific ACE inhibitors, 0.1 mM captopril and 0.1 mM lisinopril, increased the production of 17β-estradiol by ~52.2 and 56.9%, respectively, compared with the basal value. 17β-estradiol production was not greatly affected by addition of 1 µM ANG II (Fig. 3B) or 1 µM ANG I (Fig. 3C) to the incubation system with or without the presence of rabbit lung ACE and/or ACE inhibitors. These results suggest an involvement of ACE activity in the production of 17β-estradiol.

As reported for 17β-estradiol, progesterone production (basal value, 324 ± 24 pg/mg protein) was also inhibited by addition of 2.5 μU/ml rabbit lung ACE. Captopril and lisinopril, at the same concentration used for 17β-estradiol, increased progesterone production (~102 and 147%, respectively) (Fig. 4A). Contrary to the case of 17β-estradiol, addition of ANG II to the incubation medium caused an increase of progesterone production (~87%) that was nullified by addition of rabbit lung ACE. The presence of ACE inhibitors with ANG II amplified progesterone production by 245% for captopril and 293% for lisinopril, even with addition of rabbit lung ACE (Fig. 4B). These data suggest the presence of two pathways controlling progesterone production; the first involves an ACE pathway and the second ANG II independently of ACE conversion. The results were confirmed by addition of 1 µM ANG I to the incubation medium (Fig. 4C). ANG I stimulated progesterone production via ANG II conversion by endogenous ACE. The presence of rabbit lung ACE nullified the stimulus of ANG I. ACE inhibitors prevented conversion of ANG I to ANG II, obtaining an increase of

Fig. 2. Elution profile of bullfrog synthetic ANG I incubated with frog ovary membrane suspension. Two nanomoles of ANG I (retention time 15.630 min) were incubated at 37°C for 0 min (A) and 5 min (B) with 3.2 μg of ovary membrane suspension in a total volume of 20 μl in the condition described in MATERIALS AND METHODS. C: same as in B, with addition of 1 nmol of [Val⁵]ANG II (retention time 10.855 min) before injection of sample. D: incubation mixture in presence of 0.1 mM captopril. Arrows indicate peak corresponding to [Val⁵]ANG II.
progestosterone production only through inhibition of the pathway controlled by ACE.

The basal production of androgens (521 ± 22.06 pg/mg protein) remained unchanged after treatment of frog ovary tissue with rabbit lung ACE, captopril, lisinopril, ANG II, and ANG I.

The action of ANG II in regulation of ovulation appears to be through prostaglandin production. Yoshimura et al. (37) have shown in perfused rabbit ovary that the production of PGE2 and PGF2α is increased by ANG II treatment. To confirm these data in amphibians as well, PGF2α and PGF2α production was determined in frog ovary after treatment with rabbit lung ACE, captopril, lisinopril, ANG II, and ANG I.

Figure 5 shows PGE2 production, expressed as picograms per milligram protein, by frog ovary incubated in vitro. The data show the same pattern seen in the 17β-estradiol production, confirming the influence of ACE activity on PGE2 production, and no activity by ANG II (Fig. 5B) and ANG I (Fig. 5C).

The basal value of PGF2α (359 ± 17.93 pg/mg protein) remained unchanged in the presence of rabbit lung ACE, captopril, and lisinopril (Fig. 6A). ANG II (1 µM) increased the production of PGF2α (142%) without any influence of rabbit lung ACE and/or ACE inhibitors (Fig. 6B). The data were confirmed by a batch of experiments carried out with ANG I (Fig. 6C) where only ANG II, derived from direct or indirect conversion of ANG I, increased the PGF2α production. Addition of ACE inhibitors nullified the increase. These results suggest an involvement of the ANG II pathway in PGF2α production, with no influence of the ACE pathway.

All the experiments so far described used commercial rabbit lung ACE. To confirm the results obtained, the experiments were repeated using frog ovary ACE, the partial purification of which was obtained by trypsin treatment of previously purified ovarian membranes, and after centrifugation ACE activity was recovered in supernatant as described in MATERIALS AND METHODS. The amount of frog ovary ACE used in the tests was 2.5 mU/ml. Data obtained on steroidogenesis and prosta
glandin production were similar to the results using rabbit lung ACE (Figs. 3D, 4D, 5D, and 6D). Trypsin and trypsin inhibitor had no effect on the assay system.

To study whether the response of frog ovarian tissue was dependent on or influenced by the stage of the ovarian reproductive cycle, the same experiments were carried out with frog ovary tissue taken in the prereproductive period. The in vitro results suggest a different modulation of steroidogenesis and prostaglandin production by ACE and ANG II in frog ovary obtained in...
steroid and prostaglandin releases from ovary have been widely studied in *R. esculenta* during different periods of the annual breeding cycle. Each period is characterized by a different hormonal picture (15). Therefore it seemed interesting to compare the hormonal effects of ACE, ACE inhibitors, ANG I, and ANG II on ovaries of two different periods. Furthermore, we extend this comparison by examining the effects of exogenous angiotensin. For example, estradiol blocks the ovulation induced with PMSG and hCG in immature rats (22). Furthermore, ANG II stimulates prostaglandin production in perfused rat ovary, and treatment with indomethacin completely inhibits prostaglandin production, blocking ANG II-induced ovulation (37). Reports regarding change in ovary prostaglandin production in response to ACE inhibition are not available, and conflicting data have been reported about the influence of ACE inhibitors on blood prostaglandin levels (18, 20).

Steroid and prostaglandin releases from ovary have been widely studied in *R. esculenta* during different periods of the annual breeding cycle. Each period is characterized by a different hormonal picture (15). Therefore it seemed interesting to compare the hormonal effects of ACE, ACE inhibitors, ANG I, and ANG II on ovaries of two different periods. Furthermore, we...

**DISCUSSION**

The aim of the present study was to examine the role of ACE and ANG II on ovarian steroidogenesis and prostaglandin synthesis in amphibians. In ovarian tissue, ANG II has been shown to effect steroidogenesis, but there are apparent species or cell differences in the effects of exogenous angiotensin. For example, estradiol secretion by rat ovarian fragments from PMSG-stimulated rats is stimulated by ANG II (7, 25). This may be due to increased production of androgen precursors, because aromatase activity in granulosa cells from diethylstilbestrol-treated rats is unaffected by ANG II (26). ANG II also increases progesterone secretion by cultured granulosa cells from immature diethylstilbestrol-treated rats, but this small effect was not dose dependent, which makes interpretation difficult (26). In contrast, ANG II has no effect on basal progesterone secretion by cultured bovine luteal cells but inhibits LH-stimulated progesterone secretion by a mechanism that involves inhibition of cholesterol-side chain cleavage enzyme activity (31).

With regard to ACE, enzyme inhibition by captopril did not influence the secretion of estradiol and progesterone in perfused rat ovary even 20 h after hCG (24). This data is in contradiction with results reported above, because ACE inhibitors have an ANG II-antagonistic effect.

ANG II has been reported to induce ovulation, and the specific receptor antagonist of ANG II, saralasin, blocks the ovulation induced with PMSG and hCG in immature rats (22). Furthermore, ANG II stimulates prostaglandin production in perfused rat ovary, and treatment with indomethacin completely inhibits prostaglandin production, blocking ANG II-induced ovulation (37). Reports regarding change in ovary prostaglandin production in response to ACE inhibition are not available, and conflicting data have been reported about the influence of ACE inhibitors on blood prostaglandin levels (18, 20).
found very high ACE activity in frog ovary compared with other tissues of frog. This finding may be unique for lower vertebrates or amphibian species. In rats and human beings (8, 33), it has been reported that ovary ACE activity is equal to or lower than lung ACE activity. Interestingly, Daud et al. (9) have shown in rat high levels of ACE on the granulosa cells of two functionally distinct types of ovarian follicles, the atretic and developing follicles.

With regard to the hormonal profile of frog ovary, our results can be summarized as follows.

Estradiol

Data obtained in in vitro incubation of ovary tissue with ACE and ACE inhibitors suggest the involvement of ACE activity in the estradiol production, whereas ANG I and ANG II have no effect on estradiol secretion. These results are not in agreement with data reported by Pucell et al. (25), where estradiol secretion is stimulated by 1 µM ANG II in in vitro incubation of rat peripubertal ovary fragments, and by Yoshimura et al. (37) in perfused rabbit ovary. Our stimulus result with ACE inhibitors also does not agree with Peterson et al. (24), who found that no changes in estradiol secretion are observed in rat ovary stimulated with hCG and perfused with captopril.

Progesterone

The experiments performed to study the effect of ACE, ANG I, and ANG II on progesterone secretion prove that ACE activity is involved independently of the ANG II direct pathway. Indeed, the increase of progesterone production can be due to treatment with either ACE inhibitors or ANG II, and contemporaneous stimulation of both pathways leads to additional stimulations in progesterone production. The results are in disagreement with those reported for rabbit and rat by other authors, in which progesterone secretion is not remarkably influenced by ANG II (37) or by ACE inhibitors (24).

Prostaglandins

Our results show two different pathways modulating prostaglandin secretion: the ACE pathway that acts on PGE₂ and the ANG II pathway that regulates PGF₂α. Our findings and the report by Yoshimura et al. (37) of the regulation of PGF₂α production by ANG II are in agreement. The results differ, however, in PGE₂ production, which in perfused rabbit ovary is stimulated by ANG II. In the species of frog studied, ANG II has no effect and the secretion is stimulated by ACE inhibitors. This difference could be due to the different experimental system used or to differences in prostaglandin production by the amphibian ovary.

To verify whether frog ovary ACE gives the same results as commercial rabbit lung ACE, we repeated the experiments with ACE purified from ovary membranes and obtained the same results. In the literature, two hypothetical explanations are proposed to explain the lack of effect of ACE inhibition in mammalian ovarian steroidogenesis (24). First, ANG I or its fragments may provide sufficient ANG II-like activity to obscure the consequence associated with the lack of ANG II, e.g., in adrenal medulla and sympathetic and central nervous systems (11). In our experimental model this hypothesis is not supported by the data, because frog progesterone production increased only when ANG I was converted into ANG II by ACE, and treatment with ACE inhibitors had no effects on ANG I. A second explanation for the lack of effect of ACE inhibition is to consider an alternative pathway within the ovary acting independently of the classical RAS, proving an autocrine/paracrine action of ANG II on ovarian cell. It is also possible that angiotensinogen may be converted into ANG II by tissue plasminogen activators (32), cathepsin D, and ton (14) in frog ovary. Our findings agree with this hypothesis, because only ANG II, added to the incubation medium, stimulated progesterone and PGF₂α production, and no inhibition was seen with ACE inhibitors.

The importance of our findings is the evidence that ACE activity modulates estradiol, progesterone, and PGE₂ production in the frog ovary. This modulatory activity may depend on the interaction of angiotensin peptides locally produced in the frog ovary with other intraovarian regulatory peptides, including bradykinin, substance P, and LH-releasing hormone, that play important roles in the regulation of ovarian function (15, 21, 36). It has been suggested that ACE activity inactivates these peptide hormones (13), which in turn may have a direct action on ovarian steroidogenesis and modulate the production of estradiol and progesterone during different stages of the ovarian cycle.

Perspectives

Our studies suggest the existence of a different role of ACE and ANG II in the regulation of mammalian ovarian steroidogenesis and prostaglandin production in frog ovary in vitro. In mammals, ACE and ANG II play a contradictory role in ovarian reproductive processes (24). Using a frog model may help to elucidate mechanisms for paracrine regulation of mammalian ovarian steroidogenesis. Furthermore, there is recent evidence from in vitro and in vivo studies that ACE is involved not only in the regulation of blood pressure and fluid balances but also in hydrolysis of biological peptides, which play an important role in the regulation of ovarian function.

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R2096 ACE AND ANG II ACTIONS IN FROG OVARY


