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Prostaglandins and Reproduction in Male Lizard, *Podarcis sicula sicula*

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ABSTRACT The possible role of prostaglandins in the reproductive processes of the male lizard, *Podarcis sicula sicula,* was investigated. The plasma levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), progesterone, androgens, and 17β-estradiol, and the in vitro basal release of these hormones by testis, epididymis and interrental, at different phases of the annual sexual cycle, were studied. In addition, the in vivo and in vitro effects of $PGF_{2\alpha}$ and PGE_2 on steroid production were evaluated. In in vivo experiments, $PGF_{2\alpha}$ and 17β-estradiol plasma levels were highest during the refractory phase; PGE_2 values were lowest and androgens highest in fighting phase. $PGF_{2\alpha}$ injection increased 17β-estradiol in refractory phase; PGE_2 increased androgens in fighting phase and 17β-estradiol in refractory phase. In in vitro experiments, testis released the highest levels of $PGF_{2\alpha}$ and 17β-estradiol in refractory phase, and of PGE_2 and androgens in fighting phase. Interrenal released the highest levels of $PGF_{2\alpha}$, PGE_2, and 17β-estradiol in refractory phase, and of androgens in fighting phase. Epididymis released the highest values of $PGF_{2\alpha}$ and PGE_2 in mating phase. $PGF_{2\alpha}$ treatment increased 17β-estradiol release by testis and interrenals in refractory phase. PGE_2 treatment increased androgen release by testis in fighting phase and 17β-estradiol release by interrenal in refractory phase. The present data indicate that in male lizards, *P. s. sicula,* testicular androgen synthesis during the fighting phase is under the control of PGE_2, while 17β-estradiol synthesis during refractory phase is regulated in the testis by $PGF_{2\alpha}$ and in interrenal by $PGF_{2\alpha}$ and PGE_2.

In laboratory mammals, prostaglandins (PGs) of both F and E series regulate testicular activities (Tso and Lacy, '75; Abbatiello et al., '75, '76; Saxena et al., '78; Chinoy et al., '80). In fact, the production of sperm in rat and mice is lowered by PGs through the inhibition of spermatid formation (Abbatiello et al., '75, '76; Tierney et al., '79; Dev and Mangat, '82). However, the testicular steroidogenetic function is not affected by PGs in rats, since prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) do not alter cholesterol amounts and activities of the 3β- and 17β-idroxysteroid dehydrogenases (Chinoy et al., '80).

In nonmammalian vertebrates, little is known about the role of PGs in the regulation of reproductive processes. Recently it was found that a mixture of $PGF_{2\alpha}$ and PGE_2 induced the appearance of a pheromonal activity in the male gobiid *Orsinigobius punctatissimus* (Colombo et al., '91). In the frog, *Rana esculenta,* and in the newt, *Triturus carnifex,* $PGF_{2\alpha}$ and PGE_2 regulate testicular steroidogenesis during the various periods of the annual sexual cycle, suggesting the involvement of these hormones in the reproductive processes of amphibians (Gobbetti et al., '91a,b, '92; Gobbetti and Zerani, '92a). In the lizard, *Sceloropus jarrovi,* Guillette et al. ('88) found that testis and epididymis are able to synthetize $PGF_{2\alpha}$ and PGE_2.

To clarify the possible role of PGs in the reproductive processes of the male lizard, *Podarcis sicula sicula,* we compared $PGF_{2\alpha}$, PGE_2, progesterone, androgen, and 17β-estradiol plasma levels and the in vitro basal release by testis, epididymis and interrenals, at different phases of the annual sexual cycle, of this lizard; in addition, the in vivo and in vitro effects of $PGF_{2\alpha}$ and PGE_2 on the steroid release were examined.

MATERIALS AND METHODS

Animals

The reproductive cycle of the male lizard, *P. s. sicula,* living in the surroundings of Naples (Campania, Italy, 25–75 m above sea level) is here briefly described. In this lizard, the annual breeding ac-

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tivity is restricted to spring. The gonads and the secondary sexual characters (SSC) develop at the beginning of the spring and are functional until June, then a refractoriness to the still favorable ambient cues begins, followed by a complete regression of gonads and SSC (Angelini et al., '80, '81, '86). The reproductive behavior of the male is characterized by fights in March and April, and mating in May (Ciarcia et al., '86).

Adult male lizards, *P. s. sicula,* of this population were captured in April (fighting phase), May (mating phase) and June (refractory phase) 1992, transferred to laboratory terraria, maintained in ambient photothermal conditions, and fed with meal worms and fresh vegetables ad libitum, to be utilized for in vivo and in vitro studies.

In vivo experiments

In April, May and June, 45 adult male lizards (for each month) were used. The animals were divided into four groups: a) 10 lizards received a single injection of 300 ng $PGF_{2\alpha}$ (Sigma Chemical Co., St. Louis, MO) dissolved in 100 μl of saline; b) 10 lizards received a single injection of 300 ng PGE_2 (Sigma) dissolved in 100 μl of saline; c) 10 lizards received a single injection of 100 μl of saline only; d) 10 lizards were untreated. Five of each group of 10 animals were bled, after decapitation, 6 and 24 hr after treatment. Individual blood samples were collected in chilled tubes containing acetylsalicilic acid and EDTA (5 μg and 7 μg/ml of blood, respectively). After centrifugation, plasma samples were kept at −20°C until use. A further batch of five untreated lizards were bled at the beginning of the experiments (time 0 in the Figures). The times of treatment and the minimum effective dose of $PGF_{2\alpha}$ and PGE_2 were chosen after preliminary tests (data not shown).

In vitro experiments

The in vitro experiments followed previous designs of Gobbetti et al. ('93). In April, May and June, 60 adult male lizards (for each month) were used. Lizards were sacrificed by decapitation, testis, epididymis, and interrenal rapidly removed, and placed in cold Dulbecco's modified Eagle medium (DME, Sigma) containing 10 mM Hepes, 1 mg penicillin/ml, and 2 mg streptomycin/ml. For each month, 30 testes, 30 epididymis, and 120 interrenals were placed in multiple tissue culture plates (Beckton Dickinson, Clifton, NJ); each well contained one testis, or one epididymis, or four interrenals randomly chosen. Each incubation set was divided into three experimental groups (each consisting of two wells). The experimental groups were a) tissues (testis, interrenals, or epididymis) incubated with DME alone; b) tissues (testis or interrenals) incubated with DME plus $PGF_{2\alpha}$ (testis: 50 ng; interrenals: 30 ng); c) tissues (testis or interrenals) incubated with DME plus PGE_2 (testis: 75 ng; interrenals: 50 ng). The final volume of each well was 1 ml. Culture plates were wrapped in aluminum foil and incubated at 32°C. The medium of one of the two wells of each experimental group was removed after 3 hr and the other after 6 hr of incubation. The incubation medium samples were immediately stored at −20°C for later hormone determination. In addition, the experiment was repeated without tissues. Tests on five parallel incubation sets were carried out. Preliminary evidence led to our choosing the incubation conditions and the $PGF_{2\alpha}$ or PGE_2 minimum effective dose utilized in the present in vitro study (data not shown).

$PGF_{2\alpha}$ PGE_2, progesterone, androgen, and 17β-estradiol determinations

Concentrations of $PGF_{2\alpha}$ and PGE_2 were measured by radioimmunoassay in plasma and incubation medium samples as described by Gobbetti et al. ('90, '93). Determinations were carried out on duplicate plasma (100 μl) and incubation medium (500 μl) samples extracted with 5 ml of diethyl ether for 5 min. After centrifugation, organic fractions were transferred into glass tubes and evaporated to dryness under nitrogen. Extracts were resuspended in 100 μl assay buffer before assay. The recoveries of added labelled PGE_2 and $PGF_{2\alpha}$ were, respectively, 87.8 ± 0.75% and 89.4 ± 1.18%. Parallelism among the standard curve in buffer, a standard curve in incubation medium (then extracted) and a serial dilution of a single medium sample (extracted) were constant.

Concentrations of progesterone, androgens, and 17β-estradiol were measured in plasma and incubation medium samples as previously described (d'Istria et al., '74; Gobbetti et al., '90).

Intra and interassay coefficients of variation and minimum detectable doses were $PGF_{2\alpha}$, 7%, 13%, 15 pg; PGE_2, 6%, 11%, 18 pg; progesterone, 6%, 8%, 9 pg; androgens, 5%, 7%, 8 pg; 17β-estradiol, 4%, 9%, 10 pg. The $PGF_{2\alpha}$, progesterone, testosterone, and 17β-estradiol antisera were provided by Dr. G.F. Bolelli and Dr. F. Franceschetti (CNR-Physiopathology of Reproduction Service, University of Bologna, Italy) and the PGE_2 antiserum was purchased from Cayman Chemical Company (Ann Arbor, MI). Testosterone was not separated from 5α-dihydrotestosterone and, therefore, since the antiserum used

is not specific, the data are expressed as androgens. Tritiated $PGF_{2\alpha}$, PGE_2, progesterone, androgens, and 17β-estradiol were purchased from Amersham International (Buckinghamshire, England), non-radioactive $PGF_{2\alpha}$, PGE_2, progesterone, androgens, and 17β-estradiol from Sigma.

Statistics

Analysis of variance (ANOVA), followed by Duncan's multiple range test, was used to analyze the data (Duncan, '55; Sokal and Rohlf, '81). Correlation coefficients were calculated as described by Scossiroli and Palenzona ('79).

RESULTS

In vivo experiments

Plasma $PGF_{2\alpha}$ levels were higher in June ($P<0.01$) with respect to the other months; May values were higher ($P<0.01$) than April ones (Fig. 1). Plasma PGE_2 levels were lower in April ($P<0.01$) with respect to the other months (Fig. 1). Plasma progesterone levels did not change during the three examined months (data not shown). Plasma androgen levels were higher in April ($P<0.01$) with respect to the other months (Fig. 2). Plasma 17β-estradiol levels were higher in June ($P<0.01$) with respect to the other months; May values were higher ($P<0.01$) than April ones (Fig. 3). Plasma $PGF_{2\alpha}$ values were positive correlated to those of 17β-estradiol ($r = 0.84$; $N = 13$; $P<0.001$) and negatively to those of androgens ($r = -0.82$; $N = 13$; $P<0.001$); plasma androgen values were negatively correlated to those of 17β-estradiol ($r = -0.79$; $N = 13$; $P<0.001$).

Plasma steroid levels of lizards injected with saline only did not significantly change (data not shown). $PGF_{2\alpha}$ treatment induced a significant ($P<0.01$) increase of plasma 17β-estradiol at 6 and 24 hr in May and June (Fig. 3). PGE_2 induced a significant ($P<0.01$) increase of plasma androgens at 6 and 24 hr in April (Fig. 2), and a significant ($P<0.01$) increase of plasma 17β-estradiol at 6 and 24 hr in May and June (Fig. 3).

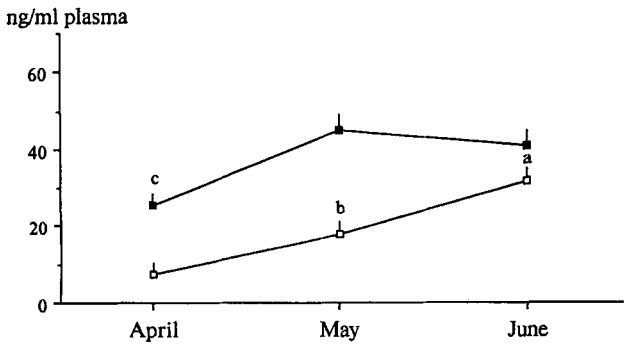

Fig. 1. $PGF_{2\alpha}$ (white squares) and PGE_2 (black squares) plasma levels of male lizard, *P. s. sicula*, during fighting (April), mating (May), and refractory (June) phases. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. April and May; b, $P<0.01$ vs. April; c, $P<0.01$ vs. May and June.

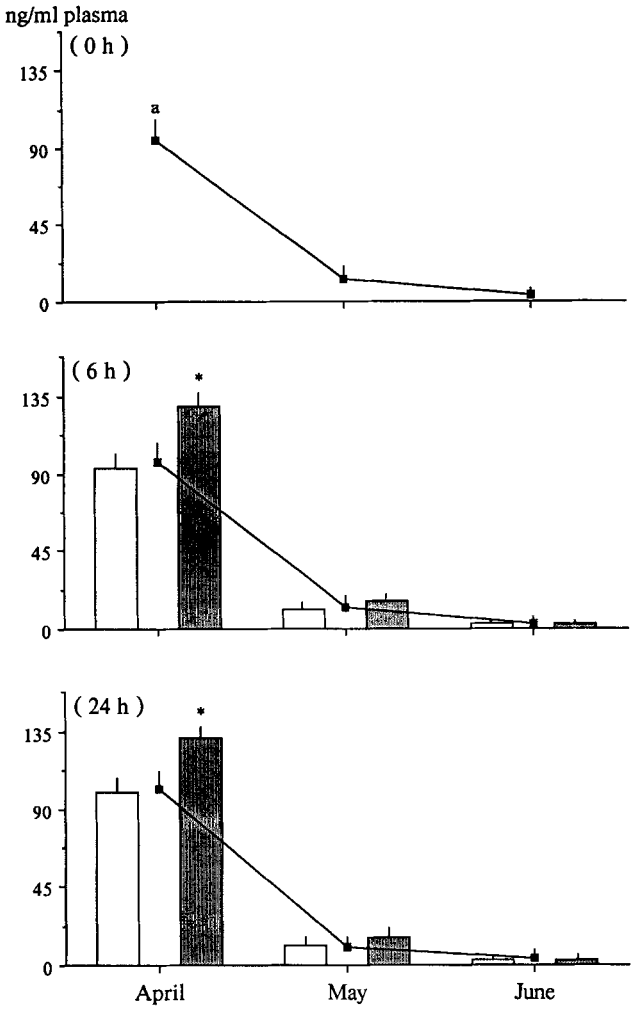

Fig. 2. In vivo effects of 300 ng $PGF_{2\alpha}$ and 300 ng PGE_2 injection on androgen plasma levels in male lizard, *P. s. sicula*, during fighting (April), mating (May), and refractory (June) phases. Experimental groups: (black squares) untreated, (white bars) $PGF_{2\alpha}$ injected, (grey bars) PGE_2 injected animals. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. May and June untreated animals; *$P<0.01$ vs. same month untreated animals.

In vitro experiments

$PGF_{2\alpha}$, PGE_2, progesterone, androgens, and 17β-estradiol were not detectable in incubation media without tissues (data not shown).

Testis

$PGF_{2\alpha}$ basal release was higher ($P<0.01$) in June with respect to the other months; the April release

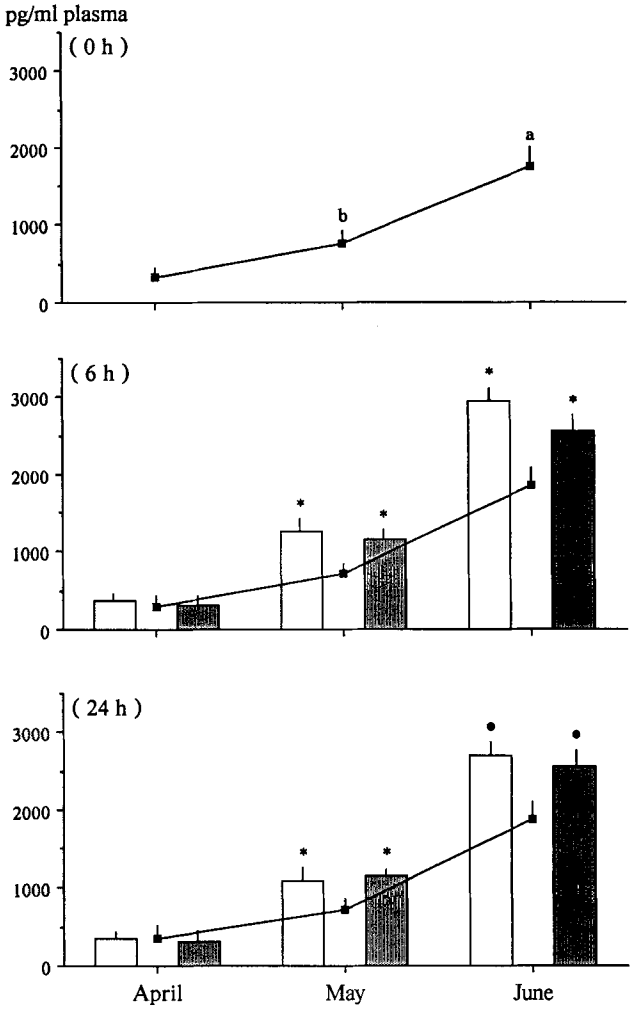

Fig. 3. In vivo effects of 300 ng $PGF_{2\alpha}$ and 300 ng PGE_2 injection on 17β-estradiol plasma levels in male lizard, *P. s. sicula*, during fighting (April), mating (May), and refractory (June) phases. Experimental groups: (black squares) untreated, (white bars) $PGF_{2\alpha}$ injected, (grey bars) PGE_2 injected animals. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. April and May untreated animals; b, $P<0.01$ vs. April untreated animals; *$P<0.01$ vs. same month untreated animals.

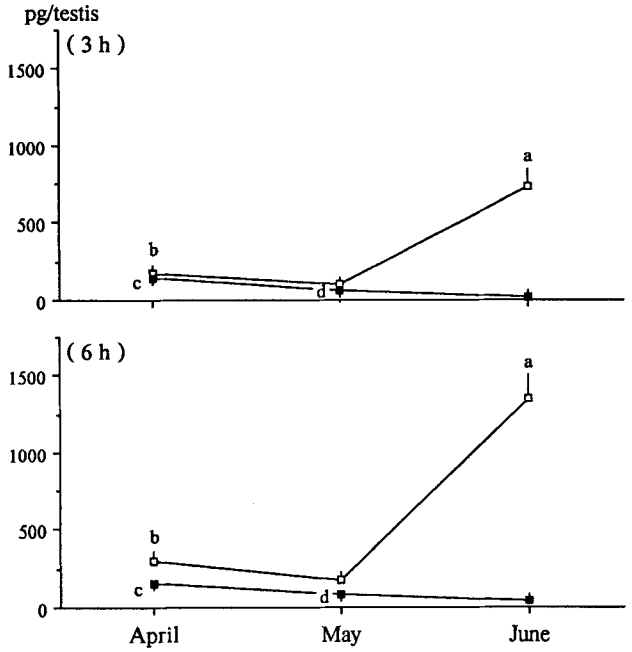

Fig. 4. In vitro basal release of $PGF_{2\alpha}$ (white squares) and PGE_2 (black squares) by testis of male lizard, *P. s. sicula*, at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. April and May; b, $P<0.01$ vs. May; c, $P<0.01$ vs. May and June; d, $P<0.01$ vs. June.

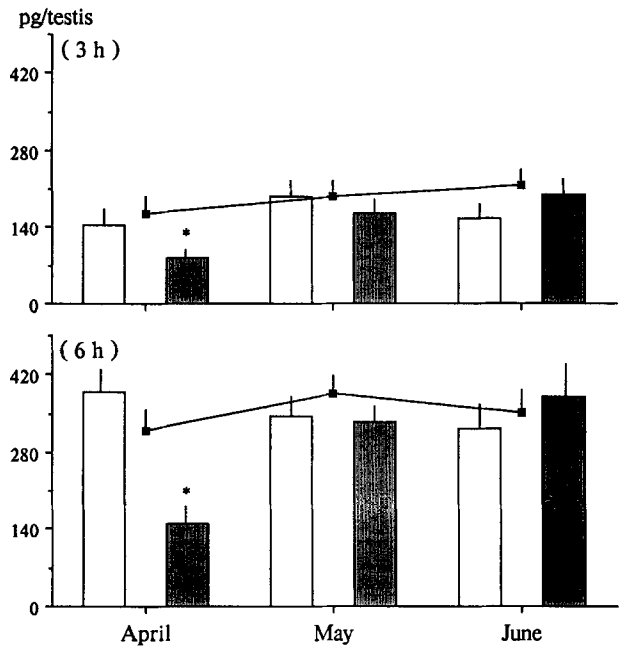

Fig. 5. In vitro effects of 50 ng $PGF_{2\alpha}$ and 75 ng PGE_2 on progesterone release by testis of male lizard, *P. s. sicula*, at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Experimental groups: (black squares) testis incubated with DME alone, (white bars) testis incubated with $PGF_{2\alpha}$, (grey bars) testis incubated with PGE_2. Each mean refers to 5 ± S.D. *$P<0.01$ vs. same month DME alone.

was higher ($P<0.01$) than the May one (Fig. 4). PGE_2 basal release was higher in April ($P<0.01$) with respect to the other months; May values were higher ($P<0.01$) than June ones (Fig. 4). Progesterone basal release did not change during the examined months (Fig. 5). Androgen basal release was higher ($P < 0.01$) in April with respect to the other months; May values were higher ($P < 0.01$) than June ones (Fig. 6). 17β-Estradiol basal release was higher ($P<0.01$) in June with respect to the other months; April values were higher ($P<0.01$) than May ones (Fig. 7). $PGF_{2\alpha}$ values were positively correlated to those of 17β-estradiol (r = 3 hr: 0.89, 6 hr: 0.87; N = 13; $P<0.001$); PGE_2 values were positively correlated

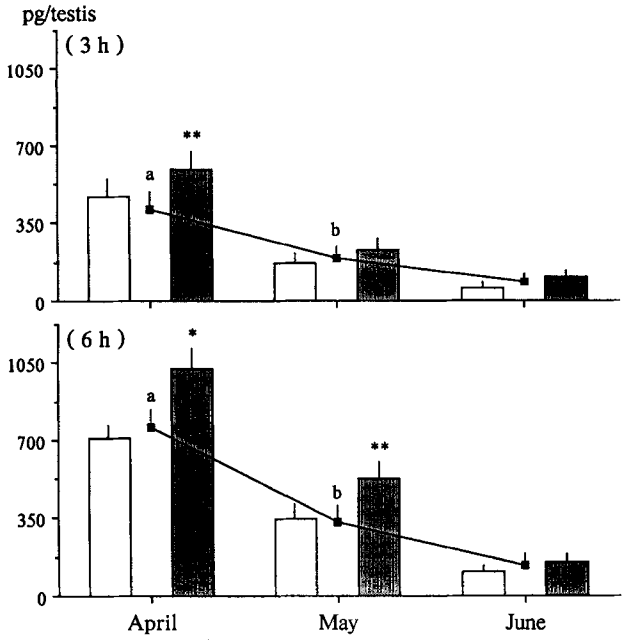

Fig. 6. In vitro effects of 50 ng $PGF_{2\alpha}$ and 75 ng PGE_2 on androgen release by testis of male lizard, *P. s. sicula*, at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Experimental groups: (black squares) testis incubated with DME alone, (white bars) testis incubated with $PGF_{2\alpha}$, (grey bars) testis incubated with PGE_2. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. May and June DME alone; b, $P<0.01$ vs. June DME alone; *$P<0.01$ vs. same month DME alone; **$P<0.05$ vs. same month DME alone.

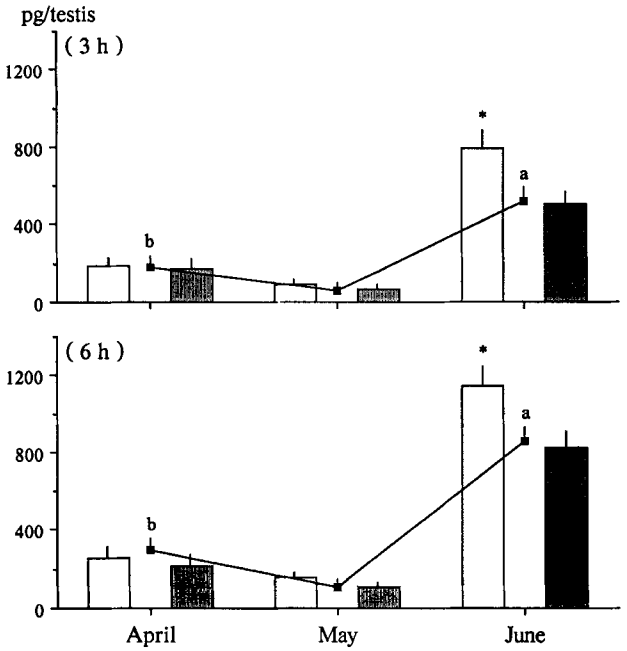

Fig. 7. In vitro effects of 50 ng $PGF_{2\alpha}$ and 75 ng PGE_2 on 17β-estradiol release by testis of male lizard, *P. s. sicula*, at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Experimental groups: (black squares) testis incubated with DME alone, (white bars) testis incubated with $PGF_{2\alpha}$, (grey bars) testis incubated with PGE_2. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. April and May DME alone; b, $P<0.01$ vs. May DME alone; *$P<0.01$ vs. same month DME alone.

to those of androgens (r = 3 hr: 0.82, 6 hr: 0.84; N = 13; $P<0.001$).

$PGF_{2\alpha}$ treatment induced a significant increase ($P<0.01$) of 17β-estradiol at 3 and 6 hr in June (Fig. 7). PGE_2 treatment induced a significant decrease ($P<0.01$) of progesterone at 3 and 6 hr in April (Fig. 5), an increase of androgens at 6 hr ($P<0.05$) in May and at 3 hr ($P<0.05$) and at 6 hr ($P<0.01$) in April (Fig. 6).

Interrenals

$PGF_{2\alpha}$ and PGE_2 basal release was higher in June ($P<0.01$) with respect to the other months (Fig. 8). Progesterone basal release was lower in April ($P<0.01$) with respect to the other months (Fig. 9). Androgens were detectable only in April (Fig. 10). 17β-Estradiol basal release was higher in June ($P<0.01$) with respect to the other months (Fig. 11). $PGF_{2\alpha}$ values were positively correlated to those of PGE_2 (r = 3 hr: 0.80, 6 hr: 0.82; N = 13; $P<0.001$) and 17β-estradiol (r = 3 hr: 0.81, 6 hr: 0.84; N = 13; $P<0.001$); PGE_2 values were positively correlated to those of 17β-estradiol (r = 3 hr: 0.85, 6 hr: 0.87; N = 13; $P<0.001$).

$PGF_{2\alpha}$ and PGE_2 treatment induced an increase of 17β-estradiol at 3 hr in May ($P<0.05$) and June ($P<0.01$), and at 6 hr ($P<0.01$) in May and June (Fig. 11).

Epididymis

$PGF_{2\alpha}$ and PGE_2 basal release was higher in May ($P<0.01$) with respect to the other months (Fig. 12); April values were higher ($P<0.01$) than June ones (Fig. 12). $PGF_{2\alpha}$ values were positively correlated to those of PGE_2 (r = 3 hr: 0.81, 6 hr: 0.83; N = 13; $P<0.001$).

DISCUSSION

This is the first paper to describe the possible involvement of $PGF_{2\alpha}$ and PGE_2 in the reproductive processes of the male lizard, *P. s. sicula*. During the various phases of reproduction, plasma $PGF_{2\alpha}$ and PGE_2 showed different values. As concerns $PGF_{2\alpha}$, this prostaglandin reached the maximum values during the refractory phase (June), characterized by the highest levels of 17β-estradiol as well. These high amounts of plasma 17β-estradiol, here reported, confirm the data found in previous works for this

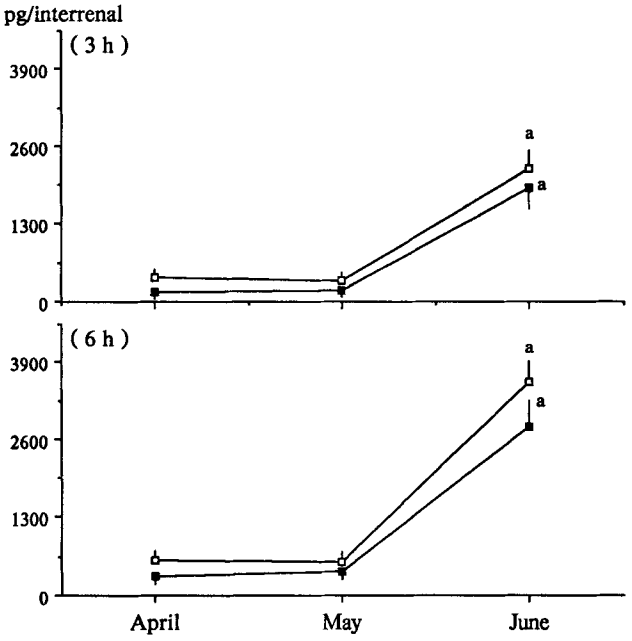

Fig. 8. In vitro basal release of $PGF_{2\alpha}$ (white squares) and PGE_2 (back squares) by interrenal of male lizard, *P. s. sicula,* at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. April and May.

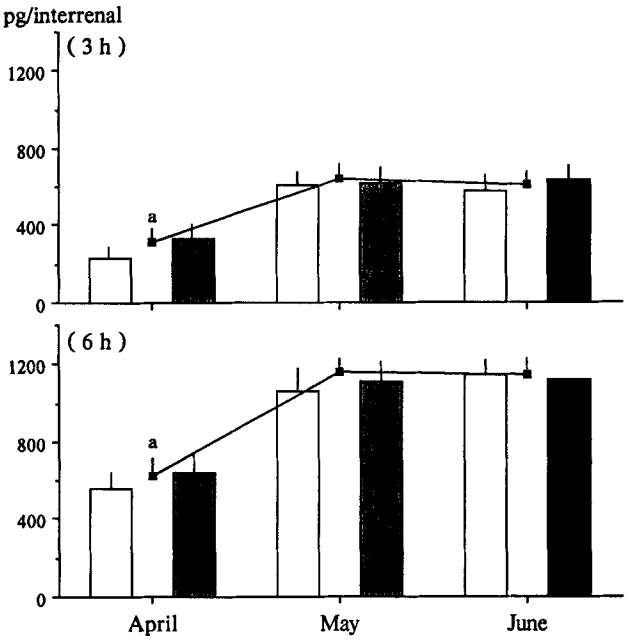

Fig. 9. In vitro effects of 30 ng $PGF_{2\alpha}$ and 50 ng PGE_2 on progesterone release by interrenal of male lizard, *P. s. sicula,* at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Experimental groups: (black squares) interrenal incubated with DME alone, (white bars) interrenal incubated with $PGF_{2\alpha}$, (grey bars) interrenal incubated with PGE_2. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. May and June DME alone.

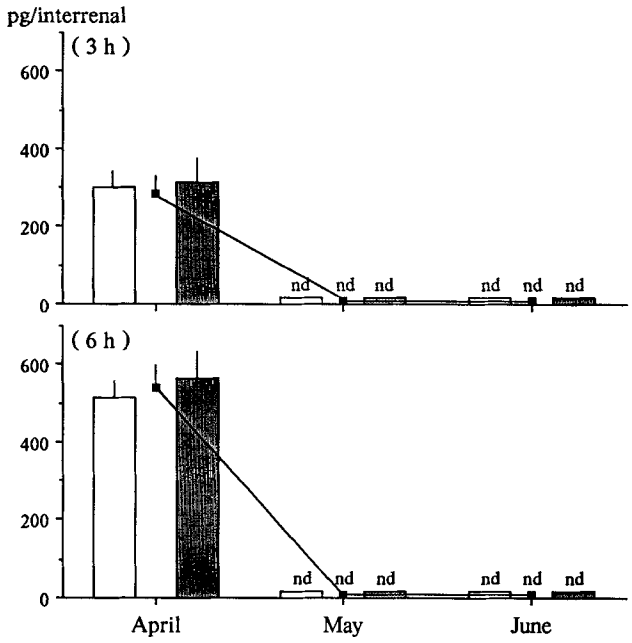

Fig. 10. In vitro effects of 30 ng $PGF_{2\alpha}$ and 50 ng PGE_2 on androgen release by interrenal of male lizard, *P. s. sicula,* at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Experimental groups: (black squares) interrenal incubated with DME alone, (white bars) interrenal incubated with $PGF_{2\alpha}$, (grey bars) interrenal incubated with PGE_2. Each mean refers to 5 ± S.D. nd, not detectable.

species (Andò et al., '92). This 17β-estradiol peak could be responsible for the interruption of the reproductive activity by acting with a negative feedback mechanism on the hypothalamo-pituitary gonadal axis, as suggested for amphibians (Pavgi and Licht, '89; Fasano et al., '91). The interruption of reproduction, when the environmental cues like temperature are still favorable, is an adaptative mechanism preventing the presence of newly-hatched lizards in early autumn as suggested by Angelini et al. ('86). In this context we recall that a $PGF_{2\alpha}$-dependent 17β-estradiol synthesis was found during the refractory phase in the anuran *R. esculenta* (Gobbetti et al., '91b, '92) and the urodele *T. carnifex* (Gobbetti et al., '91a; Gobbetti and Zerani, '92a). The relationship which links $PGF_{2\alpha}$ and 17β-estradiol is supported by the positive correlation between the plasma values of these two hormones and the in vivo stimulatory $PGF_{2\alpha}$ effects on the synthesis of 17β-estradiol found in June. The in vitro experiments, carried out on testis and interrenals, also confirm the above reported relationship; in fact, both testis and interrenals release the highest alues of $PGF_{2\alpha}$ and 17β-estradiol in June, and in the same month, $PGF_{2\alpha}$ enhanced 17β-estradiol release. These data suggest one and the same mechanism

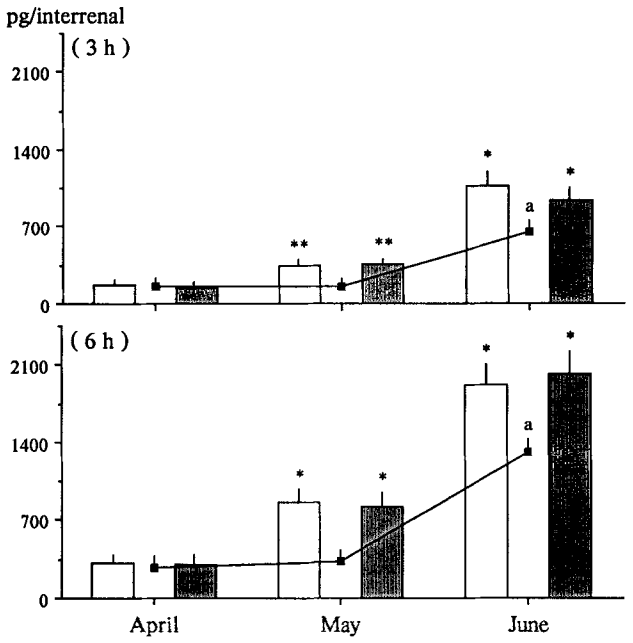

Fig. 11. In vitro effects of 30 ng $PGF_{2\alpha}$ and 50 ng PGE_2 on 17β-estradiol release by interrenal of male lizard, *P. s. sicula*, at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Experimental groups: (black squares) interrenal incubated with DME alone, (white bars) interrenal incubated with $PGF_{2\alpha}$, (grey bars) interrenal incubated with PGE_2. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. April and May DME alone; *$P<0.01$ vs. same month DME alone; **$P<0.05$ vs. DME alone.

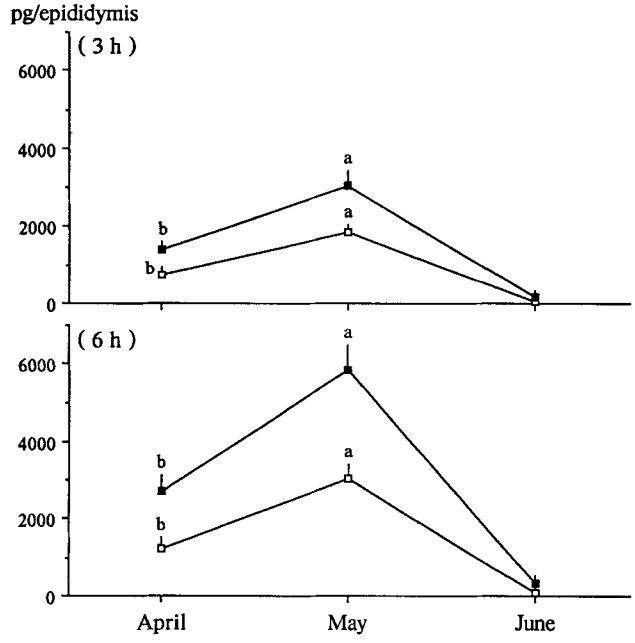

Fig. 12. In vitro basal release of $PGF_{2\alpha}$ (white squares) and PGE_2 (black squares) by epididymis of male lizard, *P. s. sicula*, at 3 and 6 hr of incubation, during fighting (April), mating (May), and refractory (June) phases. Each mean refers to 5 ± S.D. a, $P<0.01$ vs. April and June; b, $P<0.01$ vs. June.

of action for these prostaglandins in 17β-estradiol synthesis in these organs, as previously found in testis and interrenals of *R. esculenta* and *T. carnifex* (Gobbetti and Zerani, '91, '92b).

The data of plasma PGE_2 are difficult to interpret. The lowest PGE_2 levels were detected during the fighting phase, which is characterized by the highest levels of androgens, which are responsible for the aggressive behavior of the male; but surprisingly, PGE_2 in vivo treatment enhanced plasma androgens in this phase. This PGE_2-dependent androgen synthesis mirrored the results obtained in in vitro cultured testis. Also interrenals contribute to the androgen production in fighting phase, but PGE_2 failed to enhance interrenal androgen synthesis. These data indicate that PGE_2 could be implicated in favoring aggressive behavior of the male lizard through testicular androgen synthesis. However, in this context we recall that gonadotropins are the main factors which regulate testicular steroidogenesis in reptiles (Licht, '84; Lofts, '87). In addition, also PGE_2, like $PGF_{2\alpha}$, in vivo stimulated 17β-estradiol synthesis during the refractory phase, but this high 17β-estradiol production could be due to interrenal activity only. In fact, PGE_2 in vitro determined an increase in 17β-estradiol release by interrenals collected during refractory period, but not by testis. A double function for PGE_2 emerges from the present data: the regulation of testicular androgen synthesis for male fighting and the interrenal 17β-estradiol production for refractoriness.

Epididymis produced the highest amounts of $PGF_{2\alpha}$ and PGE_2 in May, during the mating phase; probably this high release of PGs by epididymis could be related to the contractility of the excurrent ducts of the male reproductive tract and to the sperm transport (Bartke, '76; Saito et al., '86; Schegel et al., '89).

As regards progesterone, the in vivo and in vitro data of this steroid at present are difficult to interpret, and further investigations are necessary to clarify the relationship between PGs and progesterone. In this context we recall that PGs regulate the progesterone synthesis also in male *T. carnifex* (Gobbetti and Zerani, '92a,c).

Summarizing, the present work suggests that in the male lizard, *P. s. sicula*, PGE_2 is involved in the regulation of testicular androgen synthesis during the fighting phase, while 17β-estradiol synthesis, responsible for the interruption of the reproductive processes, is regulated in the testis by $PGF_{2\alpha}$ and in interrenals by $PGF_{2\alpha}$ and PGE_2.

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