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 F2α (PGF2α), prostaglandin E2 (PGE2), progesterone, androgens, and 17β-estradiol, and the in vitro basal release of these hormones by testis, epididymis and interrenal, at different phases of the annual sexual cycle, were studied. In addition, the in vivo and in vitro effects of PGF2α and PGE2 on steroid production were evaluated. In vivo experiments, PGF2α and 17β-estradiol plasma levels were highest during the refractory phase; PGE2 values were lowest and androgens highest in fighting phase. PGF2α injection increased 17β-estradiol in refractory phase; PGE2 increased androgens in fighting phase and 17β-estradiol in refractory phase. In in vitro experiments, testis released the highest levels of PGF2α and 17β-estradiol in refractory phase, and of PGE2 and androgens in fighting phase. Interrenal released the highest levels of PGF2α, PGE2, and 17β-estradiol in refractory phase, and of androgens in fighting phase. Epididymis released the highest values of PGF2α and PGE2 in mating phase. PGE2 treatment increased 17β-estradiol release by testis and interrenals in refractory phase. PGE2 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 PGE2, while 17β-estradiol synthesis during refractory phase is regulated in the testis by PGF2α and in the interrenal by PGF2α and PGE2.

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 F2α (PGF2α) and prostaglandin E2 (PGE2) 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 PGF2α and PGE2 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*, PGF2α, and PGE2 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, *Scleropus jarrovi*, Guillette et al. ('88) found that testis and epididymis are able to synthetize PGF2α and PGE2.

To clarify the possible role of PGs in the reproductive processes of the male lizard, *Podarcis sicula sicula*, we compared PGF2α, PGE2, progesterone, androgen, and 17β-estradiol plasma levels and the in vitro basal release by testis, epididymis and interrenal, at different phases of the annual sexual cycle, of this lizard; in addition, the in vivo and in vitro effects of PGF2α and PGE2 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-
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<sub>2α</sub> (Sigma Chemical Co., St. Louis, MO) dissolved in 100 µl of saline; b) 10 lizards received a single injection of 300 ng PGE<sub>2</sub> (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<sub>2α</sub> and PGE<sub>2</sub> 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<sub>2α</sub> (testis: 50 ng; interrenals: 30 ng); c) tissues (testis or interrenals) incubated with DME plus PGE<sub>2</sub> (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<sub>2α</sub> or PGE<sub>2</sub> minimum effective dose utilized in the present in vitro study (data not shown).

PGF<sub>2α</sub>, PGE<sub>2</sub>, progesterone, androgen, and 17β-estradiol determinations

Concentrations of PGF<sub>2α</sub> and PGE<sub>2</sub> 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 re-suspended in 100 µl assay buffer before assay. The recoveries of added labelled PGE<sub>2</sub> and PGF<sub>2α</sub> 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<sub>2α</sub>, 7%, 13%, 15 pg; PGE<sub>2</sub>, 6%, 11%, 18 pg; progesterone, 6%, 8%, 9 pg; androgens, 5%, 7%, 8 pg; 17β-estradiol, 4%, 9%, 10 pg. The PGF<sub>2α</sub>, progesterone, testosterone, and 17β-estradiol antiserum were provided by Dr. G.F. Bolelli and Dr. F. Franceschetti (CNR-Physiopathology of Reproduction Service, University of Bologna, Italy) and the PGE<sub>2</sub> 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).

**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
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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.

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<sub>2α</sub> and 75 ng PGE<sub>2</sub> 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<sub>2α</sub>, (grey bars) testis incubated with PGE<sub>2</sub>. Each mean refers to ± 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.

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

PGF<sub>2α</sub> treatment induced a significant increase (P<0.01) of 17β-estradiol at 3 and 6 hr in June (Fig. 7). PGE<sub>2</sub> 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).

**Interrenal**

PGF<sub>2α</sub> and PGE<sub>2</sub> 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<sub>2α</sub> values were positively correlated to those of PGE<sub>2</sub> (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<sub>2</sub> values were positively correlated to those of 17β-estradiol (r = 3 hr: 0.85, 6 hr: 0.87; N = 13; P<0.001).

PGF<sub>2α</sub> and PGE<sub>2</sub> 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<sub>2α</sub> and PGE<sub>2</sub> 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<sub>2α</sub> values were positively correlated to those of PGE<sub>2</sub> (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<sub>2α</sub> and PGE<sub>2</sub> in the reproductive processes of the male lizard, *P. s. sicula*. During the various phases of reproduction, plasma PGF<sub>2α</sub> and PGE<sub>2</sub> showed different values. As concerns PGF<sub>2α</sub>, 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. nd, not detectable.

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. 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 hypothalamos-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 adaptive 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 values 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...
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 PGE₂ are difficult to interpret. The lowest PGE₂ 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, PGE₂ in vivo treatment enhanced plasma androgens in this phase. This PGE₂-dependent androgen synthesis mirrored the results obtained in vitro cultured testis. Also interrenals contribute to the androgen production in fighting phase, but PGE₂ failed to enhance interrenal androgen synthesis. These data indicate that PGE₂ 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 PGE₂, like PGF₂α, 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, PGE₂ in vitro determined an increase in 17β-estradiol release by interrenals collected during refractory period, but not by testis. A double function for PGE₂ 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 PGF₂α and PGE₂ 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*, PGE₂ 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 PGF₂α, and in interrenals by PGF₂α and PGE₂.
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LITERATURE CITED


