Stage-dependent modulation of Sertoli cell steroid production in dogfish (*Scyliorhinus canicula*)

P. Sourdain and D. H. Garnier

Groupe d'Etude de la Reproduction chez le Mâle (INSERM C/JF 91-04), Université de Rennes I,
Campus de Beaulieu, F-35042 Rennes cedex, France

Seminiferous lobules of dogfish (*Scyliorhinus canicula*) testis comprise cysts formed by steroid-producing Sertoli cells associated with germ cells at an identical stage of spermatogenesis. These lobules were isolated in four populations corresponding to lobules with spermatogonia (A), spermatocytes (B), early spermatids (C) and late spermatids (D). They were used for steroid radioimmunoassay or incubated with 22 α -hydroxycholesterol or with dibutyryl cyclic AMP (dibutyryl cAMP) to measure steroid production. Our results indicate that progesterone was the major steroid in seminiferous lobules at all stages of spermatogenesis except in lobules A. Furthermore, marked changes in the distribution of steroids were observed according to the stage of spermatogenesis; progesterone, 4-androstenedione, testosterone and 17 α -hydroxy-20 β -dihydroprogesterone concentrations were highest in lobules D, whereas dihydrotestosterone concentrations decreased during spermatogenesis. No significant stage-related change was observed for 3 α -diol and 3 β -diol. Incubation experiments revealed that the isolated seminiferous lobules at all stages can synthesize steroids from hydroxycholesterol and that lobules D have the highest basal contents of androstenedione and testosterone. Furthermore, when dibutyryl cAMP and 10 μ mol hydroxycholesterol l⁻¹ were added together to the cultures, an enhancement of the steroid secretion was observed rather than a change in synthesis. Our results also indicated that the responsiveness of the lobules to dibutyryl cAMP varies according to the stage of spermatogenesis and to the steroid assayed. Overall, this study indicated that germ cells probably markedly influence Sertoli cell steroidogenesis in the adult dogfish testis.

Introduction

The unit structure of the dogfish testis is a seminiferous lobule made up of spermatocysts, each composed of one Sertoli cell associated with 1–64 germ cells at a given stage of spermatogenesis. The linear progression of the spermatogenic wave from the area of lobule genesis to the opposite margin of the testis where spermiation occurs results in a zonation of the testis where all the spermatogenic stages are distinctly serial and can be microdissected (Sourdain *et al.*, 1990).

Chieffi and Lupo Di Prisco (1961) have previously identified testosterone, 4-androstenedione, progesterone and oestradiol in dogfish testicular extracts. Incubation of testicular explants of *Squalus acanthias* (Simpson *et al.*, 1964) or *Scyliorhinus canicula* (Kime, 1978, 1979) with radioactive steroids has suggested a preferential Δ^4 pathway of steroidogenesis and the principal metabolites identified from progesterone were testosterone, 4-androstenedione, 17 α -hydroxyprogesterone, 11-deoxycorticosterone and 21-hydroxylated steroids (Simpson *et al.*, 1964). Callard *et al.* (1985) have shown that testicular microsomes exhibit 17 α -hydroxylase, C₁₇₋₂₀ lyase and aromatase activities in *Squalus acanthias*.

The principal site of steroid synthesis in the testis of selacians is probably the Sertoli cell. Histochemical data have shown that

3 β -hydroxysteroid dehydrogenase (3 β -HSD) activity is localized in the cytoplasm of the Sertoli cell of the dogfish (Simpson and Wardle, 1967; Collenot, 1969). Dubois and Callard (1989) have demonstrated 3 β -HSD, hydroxylase/lyase and aromatase activities in Sertoli cells of *Squalus acanthias* *in vitro*. Furthermore, interstitial cells of the adult dogfish testis are neither abundant nor well differentiated (Collenot, 1969; Pudney and Callard, 1984b), whereas the Sertoli cells possess all the ultrastructural features of steroidogenic cells (Collenot and Ozon, 1964; Collenot and Damas, 1980; Pudney and Callard, 1984a; Mendis-Handagama *et al.*, 1991).

The regulation of Sertoli cell function by germ cells has been investigated in mammalian testes (see reviews by Parvinen, 1982; Sharpe, 1986; Jégou *et al.*, 1988; Skinner, 1991; Jégou, 1991). Many of the studies performed were based on the observation of stage-dependent changes in the function of Sertoli cells. In the dogfish testis, Sertoli cells also display stage-dependent variations as evidenced by the decrease in the number of oestrogen receptors (Callard *et al.*, 1985; Ruh *et al.*, 1986), the concentration of testicular androgen receptors (Cuevas and Callard, 1992) and by an increase in the concentration of androgen-binding protein (ABP) (Callard and Mak, 1988) during spermatogenesis. Moreover, we have demonstrated that the testicular content of progesterone,

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4-androstenedione, testosterone, dihydrotestosterone (DHT), 11-ketotestosterone (11 KT) and of 5 α -androstane 3 α ,17 β -diol (3 α -diol) varies in a stage-dependent manner and that there are stage-related changes of steroid responsiveness of testicular explants to dibutyryl cyclic AMP (dibutyryl cAMP) (Sourdain *et al.*, 1990).

The aim of this study was to investigate further interactions between germ cells and Sertoli cells in the testis of dogfish by measuring Sertoli cell steroids in seminiferous lobules isolated at different stages of spermatogenesis in the presence or absence of dibutyryl cAMP or of 22 α -hydroxycholesterol.

Materials and Methods

Animals

Mature dogfish (*Scyliorhinus canicula*) were obtained from the Station Biologique de Roscoff (Brittany, France). The fish were kept at the Station de Physiologie des Poissons (INRA, Rennes, France), in synthetic sea-water at $13 \pm 3^\circ\text{C}$, under a natural photoperiod.

Preparation of seminiferous lobules and culture

Dogfish were killed by section of the spinal cord. All the following operations were carried out in sterile conditions at 15°C . Testes were removed and immediately placed in Gautron's buffer (GB, pH 7.8, 890 mosmol kg^{-1} ; Gautron, 1978) supplemented with 100 μg gentamicin ml^{-1} , 50 IU penicillin ml^{-1} and 50 μg streptomycin ml^{-1} (GIBCO-BRL, France). After decapsulation, testes were cut transversely into 2 mm slices. Four major spermatogenic zones, namely, zone A (lobules with spermatogonia, stages I–VI of Mellinger, 1965), zone B (lobules with spermatocytes, stages VII–X), zone C (lobules with spermatids at early stages of spermiogenesis, stages XI–XII) and zone D (lobules with spermatids at late stages of spermiogenesis, stages XIII–XVIII), were identified and separated out from testis slices by using a dissecting microscope. These zones were then incubated in calcium- and magnesium-free GB (CMF) containing 1 mmol EDTA l^{-1} for 60, 90, 120 and 180 min, respectively. Seminiferous lobules were isolated by mechanical dissociation using stainless sieves (Bioblock, France) with mesh size of 350 μm for zone A or of 630 μm for the other zones. Single cells and cell debris were removed by collecting dissociated lobules under a continuous running of CMF–EDTA, washed twice with CMF for each step, and allowed to sediment at unit gravity for less than 15 min. Seminiferous lobules were then incubated in GB containing 86 KIU DNAase ml^{-1} (final concentration) for 10 min and washed with culture medium Leibovitz-L15 (GIBCO-BRL, France) adjusted (pH 7.8, 930 mosmol kg^{-1}) for 330 mmol urea l^{-1} and 280 mmol sodium chloride l^{-1} and supplemented with 4.6 mmol glucose l^{-1} , 20 mmol Hepes l^{-1} and 2% Ultrosor (steroid free serum substitute, IBF, France).

This dissociation procedure led to the harvesting of 8000 lobules A (90–98% purity) 5000 lobules B (75–83% purity), 10 000 lobules C (80–89% purity) and 18 000 lobules D (94–98% purity) per testis (Fig. 1). Routinely, $6\text{--}9 \times 10^3$ intact

seminiferous lobules were cultured in 6-well plates (NUNC; diameter of the well: 35 mm) for 12 h in air at 15°C , in 2 ml culture medium, in the presence or absence of dibutyryl cAMP (Sigma, France), with or without 22 α -hydroxycholesterol (Sigma). At the end of the incubation, tissues and media were separated and stored at -80°C until assayed.

Steroid analysis

Steroids. Nonradioactive steroids were purchased from Steraloids (Wilton, NH). Standard dilutions (5–1000 pg in 100 μl) were prepared in phosphate buffer from a stock methanol solution (100 μg ml^{-1}). Radioactive steroids were purchased from Amersham-France (Les Ulis, France): 2.59 TBq [1,2,6,7- ^3H]testosterone mmol^{-1} ; 2.96 TBq [1,2,6,7- ^3H]androst-4-ene-3,17-dione mmol^{-1} ; 6.66 TBq 5 α -dihydro-[1,2,5,6,7,16,17- ^3H]testosterone mmol^{-1} ; 1.95 TBq 5 α -[1 α 2 α (n)- ^3H]androstane-3 α ,17 β -diol mmol^{-1} ; 1.93 TBq 5 α -[1 α 2 α (n)- ^3H]androstane-3 β ,17 β -diol mmol^{-1} ; 4 TBq [1,2,6,7,16,17- ^3H]progesterone mmol^{-1} ; labelled 17 α -hydroxy,20 β -dihydroprogesterone was prepared from 2.07 TBq 17 α -hydroxy-[1,2,6,7- ^3H]progesterone mmol^{-1} .

Radioimmunoassay of steroids. For each assay, the recovery was estimated by the addition of 2000 d.p.m. assayed steroid before extraction. Progesterone, 4-androstenedione, testosterone, DHT, 5 α -androstane 3 β ,17 β -diol (3 β -diol) and 3 α -diol were assayed in tissues and media after extraction with a cyclohexane/ethyl acetate mixture (1/1, v/v). This was followed by separation on a celite (535) column using an isooctane–benzene mixture as eluant according to Corpechot *et al.* (1981) and Garnier (1985), whereas 17 α -hydroxy,20 β -dihydroprogesterone (17 α 20 β OHP) was analysed after dichloromethane extraction only. The assay of progesterone was performed according to Yenikoye *et al.* (1981). The mean crossreactivities of the anti-progesterone-11 α -succinyl-bovine serum albumin were 9% with 17 α -progesterone and 3% with 20 α -hydroxyprogesterone. The intra-assay coefficient of variation was 10%. Testosterone and DHT were assayed according to Garnier *et al.* (1978). The anti-testosterone-3-(O-carboxymethyl)oxime-bovine serum albumin (anti-testosterone-3-CMO–BSA) mainly crossreacted with 5 α -DHT (60%), 5 β -DHT (20%), 3 α -diol (36%), 3 β -diol (25%), epitestosterone (3.5%) and ethiocholanolone (2.8%). The intra-assay coefficient of variation was 9%. The anti-5 α -DHT-1 α -CH₂COOH–BSA crossreacted with 5 β -DHT (48%), 3 α -diol (30%), 3 β -diol (18%), testosterone (12%) and androsterone (4%). The intra-assay coefficient of variation was 16%. This antibody was also used to measure 3 α -diol concentrations. The intra-assay coefficient for this assay was 20%. 3 α -diol and 3 β -diol were assayed according to Garnier (1985). Anti-3 β -diol-7-CMO–BSA was purchased from Sigma (A-0531, lyophilised) and used according to manufacturers instructions. The intra-assay coefficient of variation was 7%. Androstenedione was assayed according to Carrié-Lemoine *et al.* (1983). The anti-4-androstenedione-11 α -hemi-succinyl-bovine serum albumin crossreacted with 3 α -diol (7%), epiandrosterone (5%) and testosterone (1.8%). The intra-assay coefficient of variation was 15%. The assay of 17 α 20 β OHP was performed as described by Fostier *et al.* (1981). The mean crossreactivities of the anti-17 α 20 β OHP-3-CMO–BSA were

Fig. 1. Transillumination of isolated seminiferous lobules: (a) lobules with spermatogonia; (b) lobules with spermatocytes; (c) lobules with early spermatids; (d) lobules with late spermatids. Arrows show contaminating seminiferous lobules. For (a–d) bar is 180 μ m.

2% with 20 β -dihydroprogesterone, 1% with 5 β -pregnan-17 α , 20 β -diol-3-one and 1% with 17 α 20 α OHP. The intra-assay coefficient of variation was 12%. Owing to the high crossreactivities of the anti-testosterone antibody (see below), the results of testosterone assay in culture media without steroid chromatography were referred to as 'androgen' concentrations. In this case, the intra-assay coefficient of variation was 7%. In the case of immunoprecipitation of free steroid from bound fraction, phosphate buffer supplemented with 8% poly(ethylene glycol) was used for the centrifugation. In each experiment, all samples were measured in the same assay to avoid interassay variations. The sensitivity of the assays are indicated in the figure legends.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) to determine significant differences between means.

Results

Steroid content of seminiferous lobules at different stages of spermatogenesis

Large differences in the respective concentrations of steroids were observed in the seminiferous lobules of the dogfish testis (Fig. 2). In all the lobules except those with spermatogonia (A), progesterone was the major steroid. As spermatogenesis progressed, an increase in the content of progesterone was observed. Lobules with late spermatids (D) showed significantly ($P < 0.005$) higher amounts of progesterone, androstenedione, testosterone and of 17 α 20 β OHP than other lobules. In contrast, DHT concentrations, which were found at very low concentrations throughout spermatogenesis, decreased from lobules A to D. No stage-dependent variations in 3 α -diol and 3 β -diol concentrations were observed.

Fig. 2. Steroid contents in the seminiferous lobules of the adult dogfish isolated at different stages of spermatogenesis. A: lobules with spermatogonia, stage IV–VI of Mellinger (1965); B: lobules with spermatocytes, stages VII–X of Mellinger, 1965; C: lobules at the earliest stages of spermiogenesis, stages XI–XII of Mellinger, 1965; D: lobules at the latest stages of spermiogenesis, stages XIII–XVII of Mellinger, 1965. Values represent the means $\pm$ SEM, $n = 6$. For lobules A and C, the sensitivity of the assay was 0.5 fg per lobule for all steroids except for progesterone (1 fg per lobule). For lobules B, the sensitivity was 0.6 fg per lobule except for progesterone (1.25 fg per lobule). For lobules D, the sensitivity was 0.15 fg per lobule except for progesterone (0.3 fg per lobule). 17 α ,20 β -OH-P: 17 α -hydroxy, 20 β -dihydroprogesterone; DHT: dihydrotestosterone; 3 α -diol: 5 α -androstane 3 α , 17 β -diol; 3 β -diol: 5 α -androstane 3 β , 17 β -diol. If not specified by brackets, D values are compared with A, B and C values. * $P < 0.05$, ** $P < 0.005$ (ANOVA).

Stage-related steroid production in vitro

Effect of 22 α -hydroxycholesterol. The synthesis of progesterone (Fig. 3a), androstenedione (Fig. 3b) and testosterone (Fig. 3c) by seminiferous lobules was studied in the presence of increasing concentrations of 22 α -hydroxycholesterol (0–200 $\mu\text{mol l}^{-1}$).

As shown above (Fig. 2), basal amounts of these steroids were highest in the lobules at the later stages of spermiogenesis. When 22 α -hydroxycholesterol was added, a dose-dependent increase of progesterone, androstenedione and testosterone synthesis was seen in all lobules, except in lobules with late spermatids (D), in which the increase of androstenedione and of testosterone concentrations was observed only in the culture media.

The biosynthesis of progesterone reached its maximum with 200 $\mu\text{mol l}^{-1}$ 22 α -hydroxycholesterol in all the categories of lobule. Progesterone concentrations were higher in tissue than in media in lobules B to D but not in lobules A in which progesterone concentrations in media and tissues were similar. When progesterone concentrations in the medium and the

tissues were added together, the maximum stimulated concentrations of this steroid, observed in the presence of 22 α -hydroxycholesterol, were higher in lobules C and B than in lobules D and A. The factor of stimulation of progesterone biosynthesis by 22 α -hydroxycholesterol decreased during spermatogenesis (Table 1) and the sensitivity of the lobules to 22 α -hydroxycholesterol necessary to elicit the half-maximal response of progesterone production (ED_{50}), decreased from lobules A to lobules D (Table 1).

The biosynthesis of androstenedione reached its highest values for 100 $\mu\text{mol l}^{-1}$ 22 α -hydroxycholesterol in lobules B, C, D and for 200 $\mu\text{mol l}^{-1}$ for the lobules A (Fig. 3b). Androstenedione concentrations were higher in tissues than in media in lobules A to C but not in lobules D, where the opposite was seen. No significant change in androstenedione concentrations was observed in the tissues of lobules D. When androstenedione concentrations in the medium and the tissues were added together, the maximum stimulated concentrations

Fig. 3. (a) Progesterone, (b) androstenedione, (c) testosterone concentrations in media (●—●) and tissues (○---○) from isolated seminiferous lobules (A: with spermatogonia; B: with spermatocytes; C: with early spermatids; D: with late spermatids), incubated for 12 h at 15°C, with increasing concentrations of 22α -hydroxycholesterol. Values represent the means $\pm$ SEM of four replicates and six dogfish. The sensitivity of the assays was 1.4 fg per lobule for progesterone and 0.7 fg per lobule for androstenedione and testosterone. * $P < 0.05$, ** $P < 0.005$ compared with the values obtained in absence of 22α -hydroxycholesterol (ANOVA).

Table 1. Characteristics of the dose-dependent curves of steroids in response to 22 α -hydroxycholesterol

Lobules	A	B	C	D
Progesterone				
Factor of stimulation	2600	1450	900	40
ED ₅₀ ($\mu\text{mol l}^{-1}$)	10	40	50	70
Androstenedione				
Factor of stimulation	3.5	4.3	2.4	1.2
ED ₅₀ ($\mu\text{mol l}^{-1}$)	120	40	20	7
Testosterone				
Factor of stimulation	2.4	4	3.8	1.4*
ED ₅₀ ($\mu\text{mol l}^{-1}$)	10	10	10	2*

ED₅₀: concentration of 22 α -hydroxycholesterol necessary to elicit the half-maximal response of steroid production. Factor of stimulation: maximum stimulated steroid concentrations divided by basal values; * in media only.

with 22 α -hydroxycholesterol increased during spermatogenesis. The stimulatory factor of androstenedione biosynthesis was higher in lobules B and A than in lobules C and D and the sensitivity of the lobules to 22 α -hydroxycholesterol increased as spermatogenesis proceeded (Table 1).

The biosynthesis of testosterone reached a maximum with 100 μmol (lobules B and C) or 200 μmol (lobules A) 22 α -hydroxycholesterol l^{-1} . In contrast to the other steroids examined, testosterone secretion was greater in the media than in the tissues for lobules A and B. Testosterone concentrations in culture media of the lobules at the latest stages of spermiogenesis (D) were slightly stimulated by 22 α -hydroxycholesterol, whereas no significant change was observed in the tissues. However, the sum of testosterone concentrations in media and tissues of lobules D was higher than in the other lobules. The stimulatory factor of testosterone biosynthesis was higher in lobules B and C than in lobules A (Table 1); the ED₅₀ value was similar for these three categories of lobule.

Effect of dibutyryl cAMP on androgens. With increasing concentrations of dibutyryl cAMP, a slight dose-dependent increase in androgen concentrations was observed (Fig. 4). The factor of stimulation was 1.4 and the ED₅₀ value was 10 mmol l^{-1} . In contrast, androgen concentrations in culture media of lobules A, B, and C were not significantly enhanced by dibutyryl cAMP (data not shown).

Effect of dibutyryl cAMP in the presence of 10 μmol 22 α -hydroxycholesterol l^{-1} . The concentrations of progesterone (Fig. 5a), androstenedione (Fig. 5b) and testosterone (Fig. 5c) were analysed in tissues and media of seminiferous lobules, incubated *in vitro* with or without increasing concentrations of dibutyryl cAMP (2.5–20 mmol l^{-1}).

Dibutyryl cAMP could induce a slight increase of steroid concentrations in the media. In lobules A only, the highest dose tested resulted in a significant increase of progesterone (about twofold) and androstenedione (about threefold) secretion in the media. In lobules B and C, dibutyryl cAMP induced an increase in the testosterone concentrations in the media, whereas progesterone and androstenedione concentrations in tissues and

Fig. 4. Androgen concentrations in media of isolated seminiferous lobules containing last stages of spermiogenesis (lobules D) incubated for 12 h at 15°C with increasing concentrations of dibutyryl cAMP. Values represent the means $\pm$ SEM of four replicates and four dogfish. The sensitivity of the assay was 0.6 fg per lobule. * $P < 0.05$, ** $P < 0.01$ compared with the values obtained in the absence of dibutyryl cAMP (ANOVA).

media were unaffected. ED₅₀ values were 4.5 mmol l^{-1} for lobules B and 10 mmol l^{-1} for lobules C and the stimulatory factors were 2.3 and 3 for lobules B and C, respectively. With 20 mmol dibutyryl cAMP l^{-1} , the maximum concentrations of testosterone were higher in the media of lobules C than in the media of lobules B. In lobules D, dibutyryl cAMP induced a dose-dependent decrease of androstenedione in tissues and the factor of inhibition was 1.7. This decrease in androstenedione concentrations in tissues was partially due to an increase of androstenedione in medium with a factor of stimulation equal to 1.3. The factor of inhibition of the androstenedione synthesis (sum of media and tissues) was 1.2. Moreover, it appeared that the concentrations of progesterone and androstenedione were higher in the tissues than in the media except for androstenedione concentrations for lobules D at 10 and 20 mmol dibutyryl cAMP l^{-1} . The concentrations of testosterone were similar in media and in tissues for lobules A and B and for lobules C between 0 and 5 mmol dibutyryl cAMP l^{-1} , and were higher in tissues than in media for lobules D.

Discussion

From this work, it appears that progesterone is quantitatively the major steroid present in isolated lobules, whereas, in a

Fig. 5. (a) Progesterone, (b) androstenedione, (c) testosterone concentrations in media (●—●) and tissues (○---○) from isolated seminiferous lobules (A: with spermatogonia; B: with spermatocytes; C: with early spermatids; D: with late spermatids), incubated for 12 h at 15°C with increasing concentrations of dibutyryl cAMP. Values represent the means $\pm$ SEM of three replicates and six dogfish. The sensitivity of the assays was 1.6 fg per lobule for progesterone and 0.8 fg per lobule for androstenedione and testosterone. * P < 0.05, ** P < 0.005, compared with the values obtained in the absence of dibutyryl cAMP (ANOVA).

previous study, testosterone was found to be the predominant steroid in tissues at all stages of spermatogenesis (Sourdain *et al.*, 1990). This difference can be explained by the fact that the steroids assayed in testicular tissues represent the sum of the steroids present in the seminiferous lobules plus those present in the interlobular compartment.

The stage-dependent variations of progesterone, androstenedione and dihydrotestosterone in the lobules observed here were similar to those observed in testicular tissues (Sourdain *et al.*, 1990). Slight differences were observed for testosterone, whereas the pattern of 3α -diol and $17\alpha 20\beta$ OHP contents during spermatogenesis were different.

The increase of progesterone, androstenedione and testosterone contents occurring during the later stages of spermatogenesis parallels the rise in the number of the Sertoli cell organelles, such as agranular endoplasmic reticulum, classically considered to be involved in steroidogenesis (Collenot and Damas, 1980; Pudney and Callard, 1984b) and also with the studies of Callard and collaborators showing an increase of microsomal 3β -HSD activity during spermatogenesis (Callard *et al.*, 1985; Dubois and Callard, 1989).

The fact that the testosterone content of the seminiferous lobules increased during the late stages of spermatogenesis suggests that there is a relationship between this steroid and spermiogenesis. This hypothesis is reinforced by the study of Callard and Mak (1988) which showed an increase of androgen-binding protein (ABP) concentrations during spermatogenesis. Moreover in rats, an increase in the concentrations of androgens (Parvinen and Ruokonen, 1982) and of the secretion of ABP (Ritzen *et al.*, 1982) are observed in stages VII–VIII of the seminiferous epithelium which were characterized by the last steps of spermiogenesis and by spermiation. Moreover, these stages are considered androgen-dependent (Sharpe *et al.*, 1988, 1990).

In the present study, the concentration of $17\alpha 20\beta$ OHP was higher in lobules collected from zone D. This is interesting, since the concentration of this steroid also increases in a teleost fish, during the period of active spermiation, in which the involvement of spermatozoa in the production of $17\alpha 20\beta$ OHP from 17α -hydroxyprogesterone was shown (Sakai *et al.*, 1989a, b; Loir, 1990).

The use of 22α -hydroxycholesterol provided not only an estimation of cholesterol side chain cleavage cytochrome P_{450} (P_{450SCC}) activity but also of the various enzyme activities in the pathways leading to progesterone (3β -HSD), androstenedione (17α -hydroxylase and C_{17-20} lyase) and to testosterone (17β -HSD). In our study, the optimum 22α -hydroxycholesterol concentration required to elicit the maximum rates of steroidogenesis (100 – $200 \mu\text{mol l}^{-1}$ depending on the steroid and on the nature of the lobule) was comparable to that required for rat Leydig cells ($100 \mu\text{mol l}^{-1}$, Risbridger *et al.*, 1986). Our results also indicate that all the categories of dogfish seminiferous lobule possess the enzymes necessary for biosynthesis of progesterone, androstenedione and testosterone from cholesterol; this is in agreement with the observation of Dubois and Callard (1989) showing that 3β -HSD and hydroxylase/lyase activities are present in Sertoli cells of *Squalus acanthias* testis at all stages of spermatogenesis. The fact that steroid biosynthesis was dramatically increased in the presence of 22α -hydroxycholesterol suggests that the rate-limiting step in steroidogenesis of

seminiferous lobules may be the delivery of cholesterol to P_{450SCC} cytochrome. Whereas this study shows that the dogfish seminiferous lobules can synthesize progesterone, androstenedione and testosterone from 22α -hydroxycholesterol, other studies have shown that the rat seminiferous tubules cannot convert cholesterol to androgens (Hall *et al.*, 1969; Cooke *et al.*, 1972) and that rat Sertoli cells cannot convert pregnenolone to progesterone (Wiebe *et al.*, 1988).

In this study, testosterone production by the lobules at the late stages of spermiogenesis was neither enhanced by 22α -hydroxycholesterol nor by dibutyryl cAMP but its contents remained largely higher in these lobules than in the other lobules. One possible explanation is that the basal steroidogenic activity of Sertoli cells in lobules D was already maximally stimulated, and therefore, could not be stimulated further. This may result from the fact that testosterone can exert an inhibitory effect on steroidogenic enzymes such as 3β -HSD, 17α -hydroxylase or 17β -HSD (Van der Molen and Rommerts, 1981) or on 3β -HSD and 17α -hydroxylase mRNA, as shown by Payne and Sha (1991). We have also observed that dibutyryl cAMP associated with $10 \mu\text{mol } 22\alpha$ -hydroxycholesterol l^{-1} induced a dose-dependent decrease of androstenedione synthesis in lobules D which was not translated into an increase of testosterone synthesis. This result is apparently in contradiction with the dose-dependent increase of 'androgen' concentrations in culture media in lobules D stimulated by dibutyryl cAMP only. One possible explanation is that dibutyryl cAMP may increase a steroid other than testosterone. This steroid may be 3α - or 3β -diol, the contents of which were not negligible in seminiferous lobules, since these steroids crossreacted with the testosterone anti-sera (36% and 25% for 3α -diol and 3β -diol, respectively). The lack of increase of progesterone concentrations in tissues and media of lobules incubated with dibutyryl cAMP and $10 \mu\text{mol hydroxycholesterol l}^{-1}$ may be because of an absence of progesterone biosynthesis or result from rapid metabolism of this steroid to other steroids, for example $17\alpha 20\beta$ OHP which was present in substantial amounts of lobules D. However, Kime (1978) and Cuevas and Callard (1992) showed that a sulfotransferase activity was present in dogfish testicular tissues. In a preliminary study, we observed that the concentrations of total androgen were similar to the concentrations of free androgen in blood plasma as well as in testicular tissues (data not shown). We therefore did not assay conjugated steroids in the present study. However, we have observed that the concentrations of conjugated $17\alpha 20\beta$ OHP were higher than free $17\alpha 20\beta$ OHP in blood plasma (data not shown). From these observations, a study of free and conjugated metabolites, from incubation experiments of isolated seminiferous lobules with radioactive precursor steroids, would be useful to clarify the *in vitro* steroidogenesis of seminiferous lobules in the dogfish.

The presence of peroxisomes in Sertoli cells of the dogfish (Mendis-Handagama *et al.*, 1991) and the dramatic increase of their volume per seminiferous lobule during the late stages of spermiogenesis (S. Mendis-Handagama, personal communication) reinforce the fact that Sertoli cells in lobules at the late stages of spermiogenesis possess their maximum steroidogenic capacity as previously suggested by several studies (Collenot and Ozon, 1964; Collenot and Damas 1980; Pudney and Callard, 1984a; Callard *et al.*, 1985; Dubois and Callard, 1989).

The responsiveness of lobules D for the secretion of androstenedione and testosterone in response to the 22 α -hydroxycholesterol or for the secretion of androgens in response to the dibutyryl cAMP were relatively low (1.2 to 1.4) and were similar to that obtained when whole testicular tissues were used (Sourdaine *et al.*, 1990). The fact that dibutyryl cAMP could not enhance testosterone secretion in culture media of lobules D incubated with 10 μ mol 22 α -hydroxycholesterol l^{-1} can be explained by the fact that the secretion of this steroid was already maximally stimulated by this dose of hydroxycholesterol. It appears that dibutyryl cAMP enhanced only the secretion of progesterone (lobules A), androstenedione (lobules A and D) or testosterone (lobules B and C), but not the synthesis of these steroids. It is possible that the production of androgens by the testis of selacians is independent, to some extent, of the pituitary. This view is supported by the fact that total hypophysectomy produces only a slight decrease in plasma testosterone concentrations in *S. canicula* (Dobson and Dodd, 1977), as observed in *Torpedo marmorata* and *T. ocellata* by Fasano *et al.* (1989). This highlights the importance of the contribution of testicular paracrine mechanisms and, in particular, the effects of germ cells for the control of steroid production.

In conclusion, this study establishes first, that the steroid content of seminiferous lobules varies according to the stage of spermatogenesis considered; second, isolated seminiferous lobules at all stages of spermatogenesis can synthesize steroids from hydroxycholesterol; third, dibutyryl cAMP enhances steroid secretion rather than steroid biosynthesis; fourth, the responsiveness of the lobules to dibutyryl cAMP varies according to the stage of seminiferous lobules and to the steroid assayed; fifth, lobules at the last stages of spermatogenesis have a higher basal steroidogenic activity than the lobules at other stages.

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