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

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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 22α-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 17α-hydroxy,20β-dihydroprogesterone concentrations were highest in lobules D, whereas dihydrotestosterone concentrations decreased during spermatogenesis. No significant stage-related change was observed for 3α-diol and 3β-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 μmol 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 spermatocytes, each composed of one Sertoli cell associated with 1-04 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 spermatiation occurs results in a zonation of the testis where all the spermatogenic stages are distinctly seried and can be microdissected (Sourdaine 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 Δ⁴ pathway of steroidogenesis and the principal metabolites identified from progesterone were testosterone, 4-androstenedione, 17α-hydroxyprogesterone, 11-deoxy cortisol androstane and 21-hydroxyted steroids (Simpson et al., 1964). Callard et al. (1985) have shown that testicular microsome exhibit 17α-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 3β-hydroxysteroid dehydrogenase (3β-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 3β-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; Puinney and Callard, 1984b), whereas the Sertoli cells possess all the ultrastructural features of steroidogenic cells (Collenot and Ozon, 1964; Collenot and Damas, 1980; Puinney 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 testosterone 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,
4-androstenedione, testosterone, dihydrotestosterone (DHT), 11-ketotestosterone (11 KT) and of 5α-androstane 3α,17β-diol (3α-diol) varies in a stage-dependent manner and that there are stage-related changes of steroid responsiveness of testicular explants to dibutylryl cyclic AMP (dibutylryl cAMP) (Sourdaine 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 dibutylryl cAMP or of 22α-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 ± 3°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 (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 Mellingier, 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 1⁻¹ 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 1⁻¹ and 280 mmol sodium chloride 1⁻¹ and supplemented with 4.6 mmol glucose 1⁻¹, 20 mmol Hepes 1⁻¹ and 2% Ultroser (steroid free serum substitute, IBB, 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, 6–9 × 10⁵ 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 dibutylryl cAMP (Sigma, France), with or without 22α-hydroxycholesterol (Sigma). At the end of the incubation, tissues and media were separated and stored at −80°C until assayed.

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 TqB [1,2,6,7-³H]testosterone mmol⁻¹; 2.96 TqB [1,2,6,7-³H]androst-4-ene-3,17-dione mmol⁻¹; 6.66 TqB 5α-dihydro-[1,2,5,6,7, 16,17-³H]testosterone mmol⁻¹; 1.95 TqB 5α-[1α,2α(n)-³H] androstan-3α,17β-diol mmol⁻¹; 1.93 TqB 5α-[1α,2α(m)-³H] androstan-3β,17β-diol mmol⁻¹; 4 TBq [1,2,6,7,16,17-³H] progesterone mmol⁻¹; labelled 17α-hydroxy,20β-dihydroprogesterone was prepared from 2.07 TBq 17α-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, testoster¬one, DHT, 5α-androstan 3β,17β-diol (3β-diol) and 3α-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 17α-hydroxy,20β-dihydroprogesterone (17α,20β(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-11α-succinyl—bovine serum albumin were 9% with 17α-progesterone and 3% with 20α-hydroxypro¬gesterone. 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)xime—bovine serum albumin (anti-testosterone-3-CMO–BSA) mainly crossreacted with 5α-DHT (60%), 5β-DHT (20%), 3α-diol (36%), 3β-diol (25%), epitestosterone (3.5%) and ethiocholanolone (2.8%). The intra-assay coefficient of variation was 9%. The anti-5α-DHT-1α-CH₃COOH–BSA crossreacted with 5β-DHT (48%), 3α-diol (30%), 3β-diol (18%), testosterone (12%) and androsterone (4%). The intra-assay coefficient of variation was 16%. This antibody was also used to measure 3α-diol concentrations. The intra-assay coefficient for this assay was 20%. 3α-diol and 3β-diol were assayed according to Garnier (1985). Anti-3β-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-11α-hemi-succinyl—bovine serum albumin crossreacted with 3α-diol (7%), epi-androsterone (5%) and testosterone (1.8%). The intra-assay coefficient of variation was 15%. The assay of 17α,20βOHP was performed as described by Fostier et al. (1981). The mean crossreactivities of the anti-17α,20βOHP–3-CMO–BSA were
2% with 20β-dihydroprogesterone, 1% with 5β-pregnan-17α, 20β-diol-3-one and 1% with 17α20β 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.

**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 17α20β 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 3α-diol and 3β-diol concentrations were observed.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) to determine significant differences between means.
Stage-related steroid production in vitro

Effect of 22α-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 22α-hydroxycholesterol (0–200 μmol l⁻¹).

As shown above (Fig. 2), basal amounts of these steroids were highest in the lobules at the later stages of spermatogenesis. When 22α-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 μmol 22α-hydroxycholesterol l⁻¹ 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 22α-hydroxycholesterol, were higher in lobules C and B than in lobules D and A. The factor of stimulation of progesterone biosynthesis by 22α-hydroxycholesterol decreased during spermatogenesis (Table 1) and the sensitivity of the lobules to 22α-hydroxycholesterol, represented by the concentration of 22α-hydroxycholesterol necessary to elicit the half-maximal response of progesterone production (ED₅₀), decreased from lobules A to lobules D (Table 1).

The biosynthesis of androstenedione reached its highest values for 100 μmol 22α-hydroxycholesterol l⁻¹ in lobules B, C, D and for 200 μmol 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

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**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 spermatogenesis, stages XI–XII of Mellinger, 1965; D: lobules at the latest stages of spermatogenesis, stages XIII–XVII of Mellinger, 1965. Values represent the means ± 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). 17α,20β-OH-P, 17α-hydroxy, 20β-dihydroprogesterone; DHT; dihydrotestosterone; 3α-diol; 5α-androstane 3α, 17β-diol; 3β-diol; 5α-androstane 3β, 17β-diol. If not specified by brackets, D values are compared with A, B and C values. *P < 0.05, **P < 0.005 (ANOVA).
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 ± 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).
with 22α-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 22α-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) 22α-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 spermatogenesis (D) were slightly stimulated by 22α-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 dibutyrly cAMP on androgens.** With increasing concentrations of dibutyrly 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 dibutyrly cAMP (data not shown).

**Effect of dibutyrly cAMP in the presence of 10 µmol 22α-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 dibutyrly cAMP (2.5–20 mmol l⁻¹).

Dibutyrly 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, dibutyrly cAMP induced an increase in the testosterone concentrations in the media, whereas progesterone and androstenedione concentrations in tissues and 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 dibutyrly 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, dibutyrly 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 dibutyrly 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 3 mmol dibutyrly 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 ± 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 (Sourdaine 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 (Sourdaine et al., 1990). Slight differences were observed for testosterone, whereas the pattern of 3α-diol and 17α20β OH activities in 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; Rudney 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 Ruukonen, 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 spermatogenesis and by spermiogenesis. Moreover, these stages are considered androgen-dependent (Sharpe et al., 1988, 1990).

In the present study, the concentration of 17α20β OH 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 spermatogenesis, in which the involvement of spermatooza in the production of 17α20β OH 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 P450 (P450SCC) activity but also of the various enzyme activities in the pathways leading to progesterone (3β-HSD), androstenedione (17α-hydroxylase and C17-20 lyase) and to testosterone (17β-HSD). In our study, the optimum 22α-hydroxycholesterol concentration required to elicit the maximum rates of steroidogenesis (100–200 μmol l⁻¹) depending on the steroid and on the nature of the lobule) was comparable to that required for rat Leydig cells (100 μmol l⁻¹), Rishridger 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 Syxalis ancanthis 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 P450SCC 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 older 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 Rommers, 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 μmol 22α-hydroxycholesterol 1⁻¹ 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 μmol hydroxycholesterol 1⁻¹ may be because of an absence of progesterone biosynthesis or result from rapid metabolism of this steroid to other steroids, for example 17α20β OH 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 17α20β OH were higher than free 17α20β OH 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; Rudney 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 22α-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 (Sourdaie et al., 1990). The fact that dibutyryl cAMP could not enhance testosterone secretion in culture media of lobules D incubated with 10 μmol 22α-hydroxycholesterol 1−1 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 selaciens 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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References

Callard GV, Pudney JA, Mak P and Canick JA (1985) Stage-dependent changes in steroidogenic enzymes and estrogen receptors during spermatogenesis in the testis of the dogfish Spalax acanthus Endocrinology 117 1328–1335
Cuevas ME and Callard GV (1992) Androgen and progesterone receptors in shark (Scylius) tests: characteristics and stage-related distribution Endocrinology 130 2173–2182
Dobson S and Dodd JM (1977) Endocrine control of the testis in the dogfish Scyliorhinus canicula L. I. Effects of a partial hypophysectomy on gravimetric, hormonal and biochemical aspects of tests function General and Comparative Endocrinology 32 41–51
Dubois W and Callard GV (1989) Role of the Sertoli cell in spermatogenesis: the Spalax tests model Fish Physiology and Biochemistry 7 221–227
Garnier DH (1985) Androgen and estrogen levels in the plasma of Pleurodeles waltl Michal, during the annual cycle. I. Male cycle General and Comparative Endocrinology 58 376–385
Hall PF, Irby DC and de Kretser DM (1969) Conversion of cholesterol to androgens by rat testes: comparison of interstitial cells and seminiferous tubules Endocrinology 84 488–496
Kime DE (1978) Steroid biosynthesis by the testis of the dogfish Scyliorhinus caniculus General and Comparative Endocrinology 34 6–17
Loir M (1990) Trout steroidogenic testicular cells in primary culture. II. Steroidogenic activity of interstitial cells, Sertoli cells, and spermatooza General and Comparative Endocrinology 78 368–398
Mellinger J (1965) Stades de la spermatogenèse chez Scyliorhinus caniculus (L.): description, données histochemiques, variations normales et experimentales Zeitchrift für Zellforschung 63 653–673
Parvinen M (1982) Regulation of the seminiferous epithelium Endocrine Reviews 3 404–417
Payne AH and Sha L (1991) Multiple mechanism for regulation of 3β-hydroxy-steroid dehydrogenase/Δ*-Δ*-isomerase. 17α-hydroxylase/C17-20 lyase cytochrome P450 and cholesterol side chain cleavage cytochrome P450 messenger ribonucleic acid levels in primary cultures of mouse Leydig cells Endocrinology 129 1429–1435
Ruh MF, Singh RK, Mak P and Callard GV (1986) Tissue and species specificity of unmasked nuclear acceptor sites for the estrogen receptor of Squalus testes Endocrinology 118 811–818
Sakai N, Ueda H, Suzuki N and Nagahama Y (1989a) Steroid production by Amago Salmon (Oncorhynchus rhodurus) testes at different developmental stages General and Comparative Endocrinology 75 231–240
Sakai N, Ueda H, Suzuki N and Nagahama Y (1989b) Involvement of sperm in the production of 17α,20β-dihydroxy-4-pregnen-3-one in the testis of spermiating rainbow trout, Salmon gairdneri Biomedical Research 10 131–138
Sharpe RM (1986) Paracrine control of the testis Clinical Endocrinology and Metabolism 15 185–207
Simpson TH, Wright RS and Hunt SV (1964) Steroid biosynthesis in the testis of dogfish (Squalus acanthias) Journal of Endocrinology 31 20–38
Sourdaine P, Garnier DH and Jégou B (1990) The adult dogfish (Scyliorhinus canicula L.) testis: a model to study stage-dependent changes in steroid levels during spermatogenesis Journal of Endocrinology 127 451–460

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