Molecular and Cellular Endocrinology, 40 (1985) 115–122
Elsevier Scientific Publishers Ireland, Ltd.

MCE 01289

Androgen stimulation of Sertoli cell function is enhanced by peritubular cells

Michael K. Skinner * and Irving B. Fritz **

C.H. Best Institute, Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G 1L6 (Canada)

(Received 20 September 1984; accepted 4 January 1985)

Keywords: androgen binding protein production; Sertoli cell-peritubular cell co-culture; peritubular cell conditioned medium factor(s).

Summary

Rates of production of androgen binding protein (ABP) declined during culture of purified Sertoli cell-enriched aggregates in serum-free minimal essential medium (MEM). This decline was partially prevented by the addition of testosterone to the medium, but not by 17β-estradiol addition. The androgen effect was greater in Sertoli cell aggregates in co-culture with peritubular cells than in Sertoli cell preparations depleted of peritubular cells. Initial rates of ABP production by purified Sertoli cell-enriched aggregates were also sustained when preparations were cultured in MEM supplemented with conditioned medium in which peritubular cells had been previously maintained. We interpret data presented to indicate that the stimulatory effects of peritubular cells on functions of Sertoli cells in co-culture, specifically sustained rates of ABP production, are mediated at least partially by component(s) synthesized by peritubular cells and secreted into the medium. We offer the hypothesis that androgens may influence Sertoli cells at two levels: (1) a direct action on Sertoli cells; (2) and indirect influence on Sertoli cells mediated by a direct action on peritubular cells, resulting in the formation of product(s) which modulate the rates of ABP synthesis.

Androgens maintain spermatogenesis in the acutely hypophysectomized rat by mechanisms which are only partially understood. Somatic cells in the seminiferous tubule are thought to respond directly to testosterone or dihydrotestosterone, whereas germinal cells do not (Fritz, 1978). Germinal cell development is dependent on specialized cytoarchitectural arrangements (Fawcett, 1975) and the unique microchemical environment (Setchell and Waites, 1975) within the seminiferous tubule. Sertoli cells are in large part responsible for the seminiferous tubule barrier which is essential for the maintenance of the microchemical environment within the adluminal compartment (Waites and Gladwell, 1982). Functions of Sertoli cells are influenced by adjacent peritubular cells, as judged by the interactions between these two cell types in co-culture (Tung and Fritz, 1980). The sustained production of androgen binding protein (ABP), a marker for the function of Sertoli cells in primary culture (Fritz et al., 1976), is enhanced by the presence of peritubular cells (Tung and Fritz, 1980; Hutson and Stucco, 1981; Mather et al., 1983). The survival of each cell type in serum-free medium is prolonged during co-culture (Tung and

* Present address: Department of Pharmacology, Vanderbilt University, School of Medicine, Nashville, TN 37232 (U.S.A.).

** To whom reprint requests should be addressed.

0303-7207/85/$03.30 © 1985 Elsevier Scientific Publishers Ireland, Ltd.

Fritz, 1980). In addition, a remodelling occurs during co-culture, resulting in the formation of a tubule-like structure having a basal lamina between layers of Sertoli cells and peritubular cells (Tung and Fritz, 1980).

Addition of androgens to the medium at the time of plating Sertoli cell-enriched preparations from testes of 20-day-old rats prevents the decline in rates of ABP synthesis which otherwise occurs during culture in basal medium (Louis and Fritz, 1977, 1979). These results have been interpreted as evidence in support of a direct response of Sertoli cells to androgens (Fritz, 1978; Ritzén et al., 1981). Interpretations offered could more readily be defended if the only cell type in the preparation were Sertoli cells. However, it is known that peritubular cells are present in Sertoli cell-enriched cultures, constituting about 5–7% of the total in our usual preparations (Fritz et al., 1975; Tung et al., 1984). Peritubular cells have androgen receptors (Verhoeven, 1980), accumulate androgens (Sar et al., 1975), and they respond to testosterone in vivo (Bressler and Ross, 1972) as well as in organ culture (Hovatta, 1972). The possibility therefore exists that contaminating peritubular cells in the preparation may respond directly to androgens, and then indirectly mediate the observed influences on ABP production by Sertoli cells.

We have recently reported that the number of peritubular cells in Sertoli cell-enriched preparations is reduced to very low levels (about 0.3%) after digestion with hyaluronidase, and this number is not increased during culture, even in the presence of serum (Tung et al., 1984). With the availability of these more purified Sertoli cell-enriched preparations, it became possible to perform reconstitution experiments to establish whether the maintenance of ABP production by Sertoli cells cultured in the presence of androgens was independent of peritubular cells. Results to be presented in this communication demonstrate that androgens permit maintenance of initial rates of ABP formation by co-cultures of Sertoli cells and peritubular cells to a greater extent than by hyaluronidase-treated populations of purified Sertoli cell-enriched preparations, and that peritubular cells in culture release into the medium component(s) which can maintain high rates of ABP production by Sertoli cells.

Materials and methods

Sertoli cell-enriched preparations from testes of 20-day-old Wistar rats were maintained in serum-free Eagle's minimal essential medium (MEM, Gibco), supplemented with non-essential amino acids, glutamine and antibiotics, under the same conditions as those previously described (Dorrington et al., 1975; Tung and Fritz, 1977). These cultures are called 'conventional preparations'. Purified preparations containing a lesser number of peritubular cells (0.3%) were obtained by digestion with hyaluronidase (Tung et al., 1984). Sertoli cells were plated at approximately 1×10^6 cells per well (1 ml) in Linbro plates.

Levels of ABP released into the medium were determined by radioimmunoassay, using rat ABP kits provided by the NICHD, National Hormone and Pituitary Program. We incubated samples at 4°C for 24 h with 30000 cpm iodinated rat ABP (Gunsalus et al., 1978) and rat ABP antibody (1:10000 final dilution) in buffer containing 2.5 mg/ml gelatin, 50 mM Tris–HCl at pH 7.5, 0.15 M NaCl, and 1 mM EDTA in 1.8 ml volume. We then added 200 μl goat anti-rabbit immunoglobulin (Sigma), 1:600 final dilution, and 1 ml polyethylene glycol buffer (132 mg/ml polyethylene glycol 4000 in 50 mM Tris–HCl, pH 7.5). After incubation for an additional 24 h at 4°C, samples were centrifuged at $2000 \times g$ for 2 h at 4°C, and the amount of radioiodinated protein in the pellet was determined. The radioimmunoassay was linear in the range from 1 to 30 ng ABP, and had a 10% coefficient of variation. The same relative levels were obtained, but absolute levels varied by over a factor of 2, in assays conducted with two different rat ABP kits provided by the NIH in June 1983 and March 1984, respectively. The ABP standard (WR-RI) was recalibrated by the NIH in the March 1984 rat ABP kit. For optimal results, the antibody provided in the March 1984 kit required a dilution of 1:10000, but the antibody provided in the June 1983 kit required a dilution of 1:6000. All ABP immunoassay data, except those shown in experiments described in Fig. 3, were obtained with the rat ABP kit supplied in March 1984.

Peritubular cells were isolated from testes of 20-day-old rats by harvesting cells released after collagenase digestion (Tung and Fritz, 1977).

Monocultures of primary peritubular cells were obtained by growing cells in MEM containing 10% calf serum to confluence for 2–3 days, and then removing the serum. Co-cultures of peritubular cells and Sertoli cells were obtained by plating both cell types in the absence of serum at various ratios of Sertoli cells to peritubular cells. Peritubular cells were plated initially at a density varying between approx. 5×10^4 cells per well to approx. 5×10^6 cells per well. Subsequently (30–45 min later), Sertoli cell-enriched aggregates (approx. 1×10^6 cells) were added to each well. The DNA contents in aliquots of each cell suspension were determined.

Serum-free conditioned medium was obtained from 72 h collection periods between days 2 and 5 after plating Sertoli cells, or at corresponding periods after removing serum from peritubular cell monocultures. Additions to the medium included testosterone (1 μM), 17β-estradiol (1 μM), or a mixture (FIRT) consisting of follicle-stimulating hormone (oFSH, NIH S-16, 100 ng/ml), insulin (5 μg/ml), retinol (0.35 μM) and testosterone (1 μM). The collected samples of medium were centrifuged at $10\,000 \times g$ for 30 min at 4°C, and the supernatant fractions were stored at −20°C until analyzed. Peritubular cell serum-free conditioned medium was concentrated on an Amicon ultrafiltration system with a YM10 membrane, molecular weight exclusion limit of 10 000, by approximately 100-fold (Skinner and Fritz, 1984). This medium contained no detectable ABP. The concentrated conditioned medium was added to cultures of Sertoli cells at 50 μl/ml, equivalent approximately to 1.5 μg protein per ml, in serum-free MEM. DNA levels were determined as previously described (Louis and Fritz, 1979).

ABP levels determined in the medium in which Sertoli cells had been maintained in monoculture are expressed as ng/μg DNA initially plated. Results within a single experiment in which Sertoli cells were in co-culture with peritubular cells are expressed as ng ABP per well. For comparisons of data in different co-culture experiments, the data were normalized by estimating the ng ABP/μg DNA of initially plated Sertoli cells.

Results

In confirmation of previous observations (Louis and Fritz, 1977, 1979), levels of ABP produced by our conventional preparations of Sertoli cell-enriched aggregates (CSC) were observed to decline during culture unless FSH and/or androgens were added at the time of plating the cells. The same phenomenon was detected in more highly purified preparations of Sertoli cell-enriched aggregates depleted of peritubular cells by digestion with hy-

TABLE 1

MODULATION OF LEVELS OF ABP PRODUCED BY VARIOUS TYPES OF TESTIS CELL PREPARATIONS IN CULTURE

Addition to basal medium	ABP (ng/μg Sertoli cell DNA)			
	Conventional Sertoli cell-enriched preparations (CSC)	Hyaluronidase-treated purified Sertoli cell-enriched preparations (HSC)	Peritubular cells (PC)	HSC plus PC in co-culture
None	8.5 ± 0.4 [a]	8.9 ± 0.2 [a]	0	12.5 ± 0.4 [b]
Testosterone (1 μM)	16.3 ± 0.4 [c]	12.1 ± 0.3 [b]	0	22.8 ± 0.5 [d]
17β-Estradiol (1 μM)	9.0 ± 0.4 [a]	8.0 ± 0.8 [a]	–	12.0 ± 0.8 [b]
FIRT	24.7 ± 0.7 [d]	23.1 ± 0.5 [d]	0	25.0 ± 0.7 [d]

All cells were cultured in serum-free MEM, as described in Materials and Methods. The components were added at the time of plating Sertoli cell-enriched or peritubular cell preparations. FIRT consists of a mixture of FSH (100 ng/ml), insulin (5 μg/ml), retinol (0.35 μM) and testosterone (1 μM). Levels of ABP released into the medium during the 72 h period beginning 48 h after plating Sertoli cells, or 48 h after removing the serum from peritubular cells, were determined by radioimmunoassay. Results shown are the mean ± SEM for quadruplicate wells in 4 separate sets of experiments ($n = 16$). Each Linbro well contained approx. 10^6 Sertoli cells and/or equivalent numbers of peritubular cells. Numbers with different superscripts are statistically different, with $P < 0.01$ as determined by a Student's t-test.

aluronidase (HSC) (Tung et al., 1984). Levels of ABP synthesized by CSC preparations cultured in the presence of testosterone (16.3 ng/μg Sertoli cell DNA/72 h, during days 3–5 after plating) were approximately double those produced by comparable CSC preparations cultured for the same periods in MEM alone or in MEM containing 17β-estradiol (Table 1). These basal levels of ABP production (8.5–9.0 ng/μg cell DNA) were approximately the same as those detected in HSC preparations (Table 1). However, HSC aggregates cultured in the presence of androgens synthesized only 12 ng ABP/μg Sertoli cell DNA/72 h (Table 1). It is unlikely that the diminished response by HSC preparations to testosterone resulted from damage elicited by prior digestion with hyaluronidase, since the HSC preparations responded as well as the CSC preparations to a mixture of FSH, insulin, retinol and testosterone (FIRT), with a production of 23–24 ng ABP/μg cell DNA/72 h (Table 1). The combination of components in FIRT has previously been reported to elicit maximal ABP synthesis by CSC preparations (Karl and Griswold, 1980). It should be noted that the levels of ABP reported in Table 1 should not be regarded as absolute amounts, since values obtained are dependent upon the cell preparation, the rat ABP immunoassay used, and the duration of culture before collection of medium. In other sets of experiments, using a different rat ABP kit, we obtained lower apparent absolute values (see Fig. 3), but the same relative effects were observed.

Peritubular cells (PC) in monoculture did not release detectable levels of ABP into the medium, regardless of whether androgens or FIRT were present (Table 1). Purified populations of Sertoli cell-enriched aggregates were plated with peritubular cells, as described in Materials and Methods (HSC plus PC in Table 1), and were cultured in the presence and absence of androgens or 17β-estradiol in the medium. In MEM alone, ABP production in the co-cultured system was greater than that observed in monocultures of Sertoli cells. Addition of testosterone to purified Sertoli cell-enriched preparations, co-cultured with equal numbers of peritubular cells, resulted in higher levels of ABP synthesis than those produced by HSC aggregates cultured in the presence of testosterone.

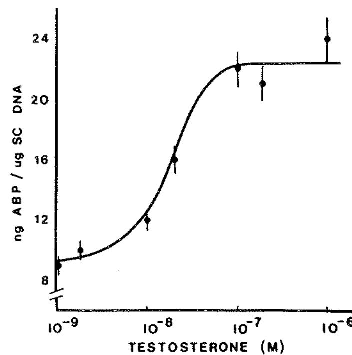

Fig. 1. Testosterone dose–response curve. Co-cultures of Sertoli cells and peritubular cells (1:1 ratio) were incubated in MEM containing various concentrations of testosterone. Medium obtained from a 72 h collection period on day 5 of culture was assayed for androgen binding protein. Data are expressed as ng ABP/μg Sertoli cell DNA (mean ± SEM) for 3 separate experiments in triplicate ($n = 9$). The testosterone concentrations indicated were those added at the time of plating, and at 48 h when the medium was changed.

The co-cultured cells (HSC + HC) maintained in the presence of estrogen had rates of ABP production indistinguishable from those observed in corresponding preparations cultured in MEM alone (Table 1).

Effects of various concentrations of testosterone on the production of ABP by Sertoli cells in the co-cultured system have been examined (Fig. 1). The amount required for half-maximal stimulation was approximately 20 nM (initial concentrations of testosterone). Levels of ABP production by co-culture maintained in the presence of 10^{-7} to 10^{-6} M testosterone (Fig. 1) were similar to those obtained in preparations cultured in the presence of FIRT (Table 1). Addition of dihydrotestosterone had the same effects as those of testosterone (data not shown).

In other experiments we examined the effects of androgens on ABP production by purified Sertoli cell-enriched preparations which were co-cultured with varying numbers of peritubular cells. The addition of 5% peritubular cells resulted in levels of ABP production similar to those observed in conventional Sertoli cell-enriched preparations cultured in the presence of testosterone (Table 1, Fig. 2). The addition of 20–50% peritubular cells resulted in maximal levels of ABP production by Sertoli cells cultured in the presence of androgen

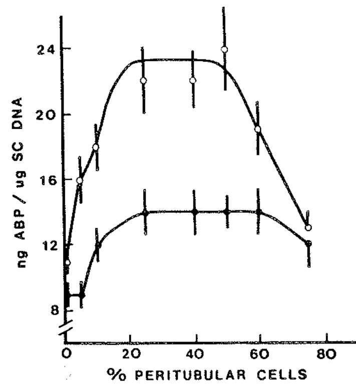

Fig. 2. Influence of the ratio of peritubular cells to Sertoli cells on ABP production. A constant number of cells in purified preparations of Sertoli cell-enriched aggregates (10^6/well) was cultured with varying numbers of peritubular cells, expressed as % peritubular cell DNA of total cell DNA. Cultures were incubated in the absence (●) or presence (○) of 1 μM testosterone at the time of plating, and replenished in the medium changed after 48 h. On day 5 of culture, medium was collected and assayed for levels of ABP. Each point, expressed as ng ABP/μg Sertoli cell DNA, is the average ± SD of 3 determinations in triplicate ($n = 9$).

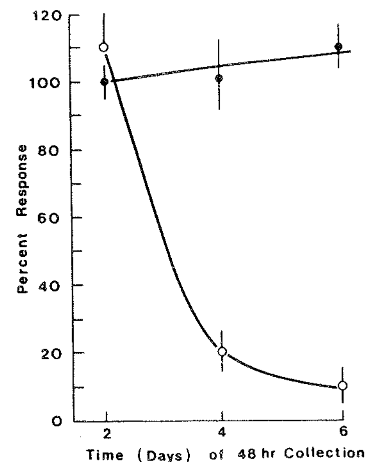

Fig. 3. Influence of peritubular cell conditioned medium on ABP production by purified preparations of Sertoli cell-enriched aggregates (HSC). Sertoli cells were cultured and incubated in the presence (●) or absence (○) of concentrated serum-free peritubular cell conditioned medium (50 μl/ml, equivalent to 1.5 μg protein per ml). After each 48 h period the medium was collected, and fresh medium of the same composition was added during the 6 days of culture. Medium was analyzed for levels of ABP. Values are expressed as 'percent response', with 100% representing the amount of ABP determined in the medium during the initial 48 h of incubation of Sertoli cells in MEM containing 50 μl/ml of peritubular cell conditioned medium. In the experiments shown, immunoassays were performed with the NIH rat ABP kit provided in June 1983, and the 100% value corresponds to 10 ng ABP/μg DNA. With the NIH rat ABP kit supplied in March 1984, the 100% value would be equivalent to 20 ng ABP/μg DNA/48 h, as described in Materials and Methods. Data are expressed as means ± SD, analyzed in triplicate in 3 separate experiments ($n = 9$).

(Fig. 2), comparable to rates of production observed when 50% peritubular cells were employed in the co-culture (Table 1). The addition of 75% or more peritubular cells to the co-culture lessened rates of ABP production by Sertoli cells cultured in the presence of testosterone (Fig. 2). In co-cultures maintained in the absence of androgens, levels of ABP produced were elevated when the peritubular cell population comprised between 10% and 60% of total cells (Fig. 2). Basal rates of ABP production were clearly elevated by the presence of peritubular cells, in confirmation of previous observations (Tung and Fritz, 1980; Hutson and Stocco, 1981; Hutson, 1983), and these rates were significantly less than those observed in corresponding preparations cultured in the presence of androgens (Fig. 2).

To determine if the enhancement of ABP production elicited by co-culture of Sertoli cells with peritubular cells could be obtained by products secreted by peritubular cells, effects of serum-free conditioned medium from these cells were examined. Peritubular cell conditioned medium, concentrated approximately 100 × by ultrafiltration as described in Materials and Methods, was added to the medium in which purified Sertoli cell-enriched aggregates were being cultured (Fig. 3). In Sertoli cell preparations maintained in basal MEM, rates of ABP production declined during successive periods of culture. In Sertoli cell preparations maintained in MEM containing 1.5 μg protein per ml of peritubular cell conditioned medium, high rates of ABP production were sustained during 6 days of culture (Fig. 3). Addition of 100 × concentrated conditioned medium prepared from MEM in which 3T3 cells had been cultured had no effects on levels of ABP production by Sertoli cells, and rates declined in a manner indistinguishable from those shown for Sertoli cells maintained in basal medium (Fig. 3, and unpublished data).

Discussion

Measurements of rates of ABP production, usually assayed by determining levels of ABP released into the medium, have provided a useful indicator of Sertoli cell functions in cultured systems, especially in relation to the hormonal responsiveness of Sertoli cells to FSH and androgens (Fritz et al., 1976; Fritz, 1978; Karl and Griswold, 1980; Louis and Fritz, 1977, 1979; Mather et al., 1983; Ritzén et al., 1981). It should be emphasized that the presence of FSH and androgens prevents the decline in rates of ABP production which occurs during culture of Sertoli cells, but that the degree of augmentation by hormones of ABP production is minimal or non-detectable during the initial 24 h culture period. The stimulation observed during subsequent days of culture may be regarded as a restoration to normal levels of ABP synthesis. Similarly, the augmentation of ABP levels in testes of hypophysectomized rats by FSH or LH administration in vivo (Vernon et al., 1974) is equivalent to a restoration of normal rates of production of ABP. The stimulation by androgens or FSH of ABP synthesis by Sertoli cells in culture thus appears to be limited to the achievement of levels initially present.

It has previously been established that co-culture of Sertoli cells with peritubular cells also helps to sustain the production of ABP, both in the presence and absence of FSH (Tung and Fritz, 1980; Hutson and Stocco, 1981). Peritubular cells alone do not synthesize ABP, or release it into the medium (Table 1), confirming previous reports (Tung and Fritz, 1980). Hutson and Stocco (1981) presented data suggesting that direct cell contact was required for the stimulation of Sertoli cells. In contrast to the findings presented here (Fig. 3), Hutson (1983) reported that addition to peritubular cell conditioned medium did not increase ABP production by Sertoli cell-enriched preparations. Similarly, stimulation of ABP production was not detected when peritubular cells and Sertoli cells were cultured in a parabiotic chamber in which the two cell types were separated by Millipore filters (Hutson, 1983). In Hutson's experiments, 'conventional' Sertoli cell-enriched preparations were cultured in medium containing 1% fetal calf serum (Hutson, 1983). Peritubular cells were probably present, since they comprise 5–7% of the population of Sertoli cell-enriched preparations not subjected to digestion with hyaluronidase (Tung et al., 1984). In MEM containing serum, peritubular cells readily divide unless inhibitors are added (Tung et al., 1980). The presence of a proliferating population of peritubular cells in the experiments reported by Hutson (1983) may have obscured the influences of added non-concentrated peritubular cell conditioned medium on ABP production by the Sertoli cell preparations employed. We cannot otherwise account for the absence of a stimulatory effect of peritubular cell conditioned medium reported by Hutson (1983), whereas it was readily detected in our experiments in which high rates of ABP production by purified Sertoli cell preparations were sustained when concentrated peritubular cell conditioned medium was added to MEM (Fig. 3).

We are evaluating the possibility that the synthesis of component(s) by peritubular cells which modulate Sertoli cell functions may be under androgenic regulation. We have examined this hypothesis by determining what changes in Sertoli cell functions can be detected when the cells are cultured in conditioned medium obtained from peritubular cells cultured in the presence and absence of androgens. In experiments presented elsewhere (Skinner and Fritz, 1984), we have observed that peritubular cell concentrated conditioned medium contains peptide(s) which stimulate Sertoli cells to produce and to release ABP, transferrin, and certain other Sertoli cell proteins. Higher levels of stimulatory factors were present in the medium of primary cultures of peritubular cells maintained in the presence of androgens than in those maintained in MEM alone, or in MEM containing estrogens (Skinner and Fritz, 1984). These data support the hypothesis that androgen actions on the seminiferous tubule are in part mediated via their actions on peritubular cells.

In the dose–response curves reported, an apparent concentration of 20 nM testosterone was required to elicit half-maximal stimulation of ABP production by Sertoli cells maintained in co-culture with equal numbers of peritubular cells (Fig. 1). This value is higher than that previously reported (4 nM), in which the testosterone levels were determined by radioimmunoassays in medium

collected at the end of the culture period (Louis and Fritz, 1979). The latter value probably provides a more accurate assessment of the true ED_{50}. The relative values obtained were the same, regardless of whether purified or conventional Sertoli cell-enriched aggregates were used. The degree of stimulation of rates of ABP production by HSC preparations by androgens was less than half of that obtained in Sertoli cell preparations cultured with equal numbers of peritubular cells (Fig. 2).

Different maximal amounts of ABP production were obtained when the ratio of peritubular cells to Sertoli cells was altered (Fig. 2). The approximately proportional increase in ABP production observed as the percentage of peritubular cells was increased to 30% is postulated to be correlated with the increased levels of peritubular cell factors released. Similarly, the greater accumulation of ABP in the androgen-treated preparations than in those cultured in the absence of testosterone (Fig. 2) is thought to be a consequence of the increased amounts of the stimulatory factor(s) released by peritubular cells in response to androgens (Skinner and Fritz, 1984). The plateau observed in ABP production when the peritubular cells comprised between 30% and 50% of the total population is not necessarily inconsistent with these views, although reasons for the failure to obtain more stimulation are not apparent. The basis for the decline in Sertoli cell activity when the peritubular cell population comprised 60–75% of the total cannot be interpreted at this time. Elevation of the ratio to 0.75 perhaps results in an alteration of Sertoli cell phenotype in culture. It is known, for example, that morphological changes of Sertoli cells occur during co-culture (Tung and Fritz, 1980), and that the histotype of Sertoli cells is altered by the nature of the substratum upon which Sertoli cells are cultured (Tung and Fritz, 1984). It is possible that the increased ratio of peritubular cells to Sertoli cells lowers the capacity of Sertoli cells to produce ABP in conjunction with these morphological changes. From an experimental vantage point, we have circumvented the problem by maintaining the ratio between 0.3 and 0.5 to obtain maximal rates of ABP production in androgen-stimulated preparations. Less ambiguous results were obtained when transferrin production was used as the indicator for Sertoli cell functions, and varying concentrations of peritubular cell conditioned medium were added to purified preparations of Sertoli cell-enriched preparations. This resulted in a proportional increase in rates of transferrin production by Sertoli cells, without evidence of inhibition at concentrations up to 80% of peritubular cell conditioned medium (Skinner and Fritz, 1984).

The possible role of peritubular cell–Sertoli cell interactions in the maintenance of the structure and functions of the seminiferous tubule in vivo remains to be delineated. We speculate that these two somatic cells of the seminiferous tubule form a functional unit which plays an integral role in the maintenance of the cytoarchitectural arrangements and the biochemical environment required for spermatogenesis to proceed. This functional unit may be analogous to that observed in other mesenchymal–epithelial cell interactions under steroidal regulation, such as in prostate (Cunha et al., 1983) and the mammary gland (Kratochwil, 1969). In the case of Sertoli cells, however, the regulation by FSH of many activities appears to be independent of androgen actions, and the influences of FSH are not dependent on the presence of peritubular cells (for review, see Fritz, 1978). Consequently, Sertoli cell functions appear to be regulated by FSH directly, by androgens directly, and indirectly by peritubular cell products whose rates of production are modulated by androgens. These and other possible influences of somatic cell interactions within the seminiferous tubule on germinal cell development have recently been reviewed (Fritz et al., 1984; Fritz, 1984).

Acknowledgements

We thank Kathy Karmally and Donna McCabe for excellent technical assistance. This work was supported by grants from the Canadian Medical Research Council, with fellowship support to M.K.S.

References

Bressler, R.S. and Ross, M.H. (1972) Biol. Reprod. 6, 143–159.
Cunha, G.R., Ghung, L.W.K., Shannon, J.M., Tanuchi, O. and Fujii, H. (1983) Recent Progr. Hormone Res. 39, 559–595.
Dorrington, J.H., Roller, N.F. and Fritz, I.B. (1975) Mol. Cell. Endocrinol. 3, 57–70.

Fawcett, D.W. (1975) in: Handbook of Physiology: Male Reproductive System, Vol. V(7), Eds.: R.O. Greep and D.W. Hamilton (Am. Physiol. Soc., Washington, DC) pp. 21–55.
Fritz, I.B. (1978) in: Biochemical Actions of Hormones, Vol. V, Ed.: G. Litwack (Academic Press, New York) pp. 249–281.
Fritz, I.B. (1984) in: Third European Workshop on Molecular and Cellular Endocrinology of the Testis, Ed. J.M. Saez (INSERM, Paris) in press.
Fritz, I.B., Louis, B.G., Tung, P.S., Griswold, M.D., Rommerts, F.G. and Dorrington, J.H. (1975) Curr. Topics Mol. Endocrinol. 2, 367–382.
Fritz, I.B., Rommerts, F.G., Louis, B.G. and Dorrington, J.H. (1976) J. Reprod. Fertil. 46, 17–24.
Fritz, I.B., Skinner, M.K. and Tung, P.S. (1984) in: VI Serono Workshop on Development and Function of the Reproductive Organs, Ed.: A. Eshkol (Raven Press, New York) in press.
Gunsalus, G.L., Musto, N.A. and Bardin, C.W. (1978) Science 200, 65–66.
Hovatta, O. (1972) Z. Zellforsch. Mikrosk. Anat. 131, 299–308.
Hutson, J.C. (1983) Endocrinology 112, 1375–1381.
Hutson, J.C. and Stocco, D.M. (1981) Endocrinology 108, 1362–1368.
Karl, A.F. and Griswold, M.D. (1980) Biochem. J. 186, 1001–1003.
Kratochwil, K. (1969) Dev. Biol. 20, 46–71.
Louis, B.G. and Fritz, I.B. (1977) Mol. Cell. Endocrinol. 7, 9–16.
Louis, B.G. and Fritz, I.B. (1979) Endocrinology 104, 454–461.
Mather, J.P., Gunsalus, G.L., Musto, M.A., Cheng, C.Y., Parvinen, M., Wright, W., Perez-Infante, V., Margioris, A., Litta, A., Becker, R., Krieger, D.T. and Bardin, C.W. (1983) J. Steroid Biochem. 19, 41–51.
Ritzén, E.M., Hansson, V. and French, F.S. (1981) in: The Testis, Eds.: H. Burger and D. de Kretser (Raven Press, New York) pp. 171–194.
Sar, M., Stumpf, W.E., McLean, W.S., Smith, A.A., Hansson, V., Nayfeh, S.N. and French, F.S. (1975) Curr. Topics Mol. Endocrinol. 2, 311–319.
Setchell, B.P. and Waites, G.M.H. (1975) in: Handbook of Physiology: Male Reproductive System, Vol. V(7), Eds.: R.O. Greep and D.W. Hamilton (Am. Physiol. Soc., Washington, DC) pp. 143–172.
Skinner, M.K. and Fritz, I.B. (1984) Proc. Natl. Acad. Sci. (U.S.A.) (in press).
Tung, P.S. and Fritz, I.B. (1977) In: Techniques of Human Andrology, Ed.: E.S.E. Hafez (Elsevier/North-Holland Biomedical Press, Amsterdam) pp. 125–146.
Tung, P.S. and Fritz, I.B. (1980) Biol. Reprod. 23, 207–217.
Tung, P.S. and Fritz, I.B. (1984) Biol. Reprod. 30, 213–229.
Tung, P.S., Lacroix, M. and Fritz, I.B. (1980) Biol. Reprod. 22, 1255–1261.
Tung, P.S., Skinner, M.K. and Fritz, I.B. (1984) Biol. Reprod. 30, 199–211.
Verhoeven, G. (1980) J. Steroid Biochem. 13, 469–474.
Vernon, R.G., Kopec, B. and Fritz, I.B. (1974) Mol. Cell. Endocrinol. 1, 167–187.
Waites, G.M.H. and Gladwell, R.T. (1982) Physiol. Rev. 62, 624–671.