Effect of uncoupling treatments on FSH-induced hyperpolarization of immature rat Sertoli cells from Sertoli cell-enriched cultures*

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Summary. Sertoli cell-enriched cultures isolated from immature rat testes by enzymic treatments were investigated by intracellular microelectrode recordings. The hyperpolarization of cells induced by FSH was independent of the age of the rats (7–37 days) and was unchanged by exposure to a hormone-free medium or to a glycine buffer of pH 3. It was reduced by treatments which decreased the electrical coupling between cells either by an increase of intracellular calcium [i.e. calcium ionophore (A 23187, 5×10^{-6} M), general anaesthetic (heptanol, 3.5 mM) and uncoupler of oxidative phosphorylations (carbonylcyanide m-chlorophenylhydrazone-CCmP, 10^{-6} M)] or by a decrease of extracellular calcium [i.e. 0Ca + EGTA (1 mM) medium]. These effects were partly or totally reversed by a recovery period in a drug-free medium. Similar results were obtained by an exposure to trypsin (0.05%) followed by a second mechanical dispersion, but new cell hyperpolarization was induced by a new exposure to FSH. This electrophysiological study suggests an initial effect of FSH on the junctional complex between Sertoli cells, then the control by calcium of this complex.

Keywords: Sertoli cells; FSH; electrophysiology; A 23187; heptanol; membrane permeability; calcium; rat

Introduction

The Sertoli cells of the testes are involved in spermatogenesis. These cells provide a nutritional and mechanical support for the germ cells (Saez *et al.*, 1985). They are exocrine cells which secrete a K^+ -enriched fluid into the lumen of seminiferous tubules (see review: Waites & Gladwell, 1982).

Follicle-stimulating hormone (FSH) elicits a number of morphological and biochemical events in the Sertoli cells of immature testes. An electrophysiological study performed on cultured Sertoli cells showed that FSH induces the hyperpolarization of the cell (Joffre & Roche, 1988) and the results suggested that FSH, by means of cAMP-dependent mechanisms, simultaneously increases Na/K pump activity and promotes Na/Ca influx through an ionic process, blocked by cobalt. This, in turn, regulates the intracellular concentration and the exit of K^+ from the cell through a K^+ channel, blocked by quinidine. Such an electrophysiological study corresponds closely with the radioisotopic and chemical results obtained in the whole seminiferous tubules concerning the exocrine function of Sertoli cells (see review: Waites & Gladwell, 1982).

Tight and gap junctions occur between Sertoli cells in the mature rat testis (Dym & Fawcett, 1970). Their formation is a prerequisite for the onset of fluid secretion (Waites & Gladwell, 1982) which starts between 15 and 19 days of age in the rat (Vitale *et al.*, 1973). This junctional complex remains unchanged even after hypophysectomy (Setchell, 1970; Johnson, 1970; Gilula *et al.*, 1976;

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Hagenas *et al.*, 1978). These junctional complexes also occur on monolayer cultures of Sertoli cells, isolated from rat testes and cultured in medium containing FSH (Solari & Fritz, 1978; Hadley *et al.*, 1985).

Studies of electrical coupling between cells have been performed on several exocrine glands (i.e. exocrine pancreas, salivary and lacrimal glands). They showed that messengers (such as hormones and drugs) which affect the functional state of the gap junctions linking neighbouring cells modify the recordings obtained by the microelectrode technique (Iwatsuki & Petersen, 1978a, b; Petersen, 1980; Ginsborg & House, 1980; Loewenstein, 1981; Poisner & Trifaro, 1985).

We have measured the membrane potential on Sertoli cells isolated from testes of immature rats, exposed to FSH before different uncoupling treatments. We have also studied the age-dependence of FSH and the effects of FSH deprivation. Preliminary accounts of this work have been published in abstract form (Roche & Joffre, 1985, 1986).

Materials and Methods

Preparation of enriched Sertoli cells and culture. The Sertoli cells were isolated from the testes of 7–37-day-old rats (Wistar). Two procedures were successively used for the isolation and culture of the cells: Procedure 1, modified from that of Welsh & Wiebe (1975), and Procedure 2, modified from that of Verhoeven *et al.* (1979). Both procedures have been described in detail (Joffre & Roche, 1988). For both procedures the cells were treated with collagenase (Boehringer Mannheim France, Meylan, France) and pancreatin (Grade VI: Sigma, St Louis, MO, USA).

For Procedure 1, the suspensions containing the tubule fragments were dispersed by being slowly passed 5–10 times (depending upon the age of the rats) through a syringe needle (17 gauge). The Sertoli cells were separated from the other cells by centrifugation through a gradient of Percoll (Pharmacia France; Les Ulis, France) by the procedure used for Leydig cells (Joffre *et al.*, 1984).

For Procedure 2, the mass of reaggregated tissues, containing peritubular cells and tubule fragments, were washed 5 times by gently shaking by hand to remove the isolated tubular fragments. Then, the washed tubules were dispersed according to Procedure 1.

The washed cells were resuspended in MEM medium (Eurobio, MEM 2116, Paris, France) (Procedure 1) or RPMI (Flow Laboratories SA Asnières, France) (Procedure 2). They were cultured in 35 mm plastic Petri dishes (Falcon 3001 or Nunclon), according to Steinberger *et al.* (1975) (Procedure 1) or Verhoeven *et al.* (1979) (Procedure 2).

Culture media. Two culture media were used.

For Procedure 1, Medium MEM (Eurobio) was supplemented by L-glutamine (2 mM; Sigma), sodium pyruvate (1 mM; Sigma), sodium bicarbonate (20 mM; Eurobio Grand Island, NY, USA), and fetal calf serum (10%; Gibco Laboratories Inc, Grand Island, NY, USA).

For Procedure 2, RPMI 1640 medium (Flow Laboratories) (including 20 mM-Hepes) was supplemented by L-glutamine (4.8 mM; Flow Laboratories) and sodium bicarbonate (20 mM; Eurobio).

Streptomycin sulphate (100 µg/ml; Gibco) and penicillin G (100 i.u./ml; Gibco) were added to both media. Culture media were changed at 2-day intervals. All culture dishes were incubated at 34°C, in a humidified incubator, in an atmosphere of 5% CO₂.

Intracellular recordings. Intracellular recordings of the membrane potential were performed by the technique of Joffre *et al.* (1984). The membrane potentials were recorded between the intracellular microelectrode fixed in a nacelle filled with 3 M KCl and an Ag/AgCl reference electrode (World Precision Instruments New Haven, CT, USA). The glass capillary microelectrodes (tip diameter 0.1–0.2 µm) were filled with 2 M-potassium citrate (pH 7.2) and had a resistance ranging between 50 and 100 MΩ.

Potentials were recorded with a WPI 707 microprobe System (World Precision Instruments) directly on the screen of an oscilloscope (Tektronix 5115, Beaverton, OR, USA).

The standard bathing solution used during the recordings was Earle's solution containing (mM): Na⁺ 121.7; K⁺ 5.4; Ca²⁺ 1.8; Mg²⁺ 0.8; Cl⁻ 125.3; PO₄H₂⁻ 0.9; SO₄²⁻ 0.8; glucose 11 and Hepes 5.

This solution was adjusted so that pH was 7.4, osmolarity was 290–300 mosmol and temperature was 34 ± 1°C.

Experimental procedures. The recordings were performed from Day 3 of culture onwards. FSH or uncoupling drugs were dissolved in the culture medium, during the incubation, or in the Earle's solution, during the electrical measurements.

All values are expressed either as individual values or as means ± s.e.m. The statistical significance of the difference between the means was established using Student's *t* test.

Hormone and drugs. Ovine FSH (NIADDK-oFSH-S₁₅ and -S₁₆) was provided by the NIAMDD Pituitary Hormone Distribution Program. Calcium ionophore (A 23187) (Boehringer-Mannheim) (stock solutions in methanol: 2 × 10⁻³ M), carbonylcyanide m-chlorophenyl hydrazine (CCmP) (Sigma) heptanol (Merck, Darmstadt, FRG),

ouabain (Nativelle, Paris, France) and trypsin (Sigma) were dissolved in the culture medium during the incubation or in the Earle's solution during the electrophysiological measurements.

Results

Morphology of the Sertoli cell-enriched cultures

Two types of Sertoli cell-enriched cultures were used to explore the electrophysiology of Sertoli cells and the FSH stimulation. In both, the cell aggregates attached themselves to the plastic dishes and flattened out. In Procedure 1 they were surrounded by peritubular myoid cells, leading to the formation of nodules. Such myoid cells were prevented by the Procedure 2 in which the Sertoli cells formed a cell monolayer, having the morphological features of a thin epithelium, as previously described (Dorrington *et al.*, 1975; Steinberger *et al.*, 1975; Solari & Fritz, 1978; Hadley *et al.*, 1985; Roche, 1986; Joffre & Roche, 1988). Some germ cells were still present in both culture types. They adhered to the apical surface of the monolayer (Hadley *et al.*, 1985; Joffre & Roche, 1988) and were largely eliminated by changing the culture medium. The remaining germ cells and the Leydig cells were always discarded from the experiments, when the Sertoli cells were impaled with the micro-electrode, under phase-contrast microscopic control. Thus, only Sertoli cells were tested throughout this experiment.

Irreversibility of the effect

The membrane potential of FSH-stimulated cells did not return to the control values when the stimulus was removed by incubating the cells in a hormone-free medium for periods of 24–72 h (Fig. 1).

Fig. 1. Recovery period of FSH-induced hyperpolarization after a 24-h exposure to $2.9 \mu\text{g}$ oFSH-S16/ml. The cells were isolated from 17-day-old rats by Procedure 2 (see 'Materials and Methods') and cultured for 3–7 days before treatment. Each point represents the mean potential $\pm$ s.e.m. for the no. of impaled cells indicated. Values were obtained from 3 different cultures. The control medium without FSH was changed every day.

Similar results were obtained when a low-pH glycine buffer (50 mM-glycine, 150 mM-NaCl; pH 3; 4°C) was added for 4 min to the Petri dishes, to remove the remaining membrane-bound FSH (Bernier & Saez, 1985) (-37.0 ± 0.6 ; $n = 51$, $P > 0.05$).

When maximal FSH stimulation was followed by another 24 h exposure to the hormone, the membrane potential did not become higher (-35.5 ± 1.3 mV; $n = 13$, $P > 0.05$).

Influence of the age of rats on the FSH induced hyperpolarization

The responsiveness of Sertoli cells to FSH decreases during the prepubertal maturation of rats (Steinberger *et al.*, 1978; Means *et al.*, 1980; Rich *et al.*, 1983). Since, *in vivo*, the effect of FSH is age-dependent, the magnitude of the membrane potential of unstimulated Sertoli cells and the FSH-induced hyperpolarization were examined in Sertoli cell-enriched cultures from the testes of 7–37-day-old rats (i.e. before, during and after the blood–testis barrier is established). Whatever the age of the rats, the membrane potential of unstimulated Sertoli cells and the FSH-induced hyperpolarization did not significantly change (Fig. 2).

Fig. 2. The effect of age of the rats on the membrane potential of unstimulated cells (●) and cells previously incubated for 24 h with FSH (2.9 µg oFSH-S16/ml) (○). The cells were isolated by Procedure 1 (see 'Materials and Methods') and cultured for 3 days before treatment. Each point represents the mean potential $\pm$ s.e.m. for the number of impaled cells indicated. Values were obtained from 3 different cultures. ∇ , Period when the blood–testis barrier is being established (Vitale *et al.*, 1973).

Effects of uncoupling treatments

In several tissues, the intracellular calcium concentration is known to control the junctional permeability between neighbouring cells. A reduction in intracellular coupling can be caused by increasing the intracellular calcium to relatively high levels or by reducing the extracellular calcium concentration with EGTA (Loewenstein *et al.*, 1967; Délèze, 1970; De Mello, 1975; Rose & Loewenstein, 1975, 1976; Spray & Bennett, 1985). To assess whether the junctional communications between Sertoli cells may modulate the FSH-induced hyperpolarization, monolayer cultures were exposed to different treatments which are known to reduce or abolish the electrical coupling between the cells.

Increased intracellular calcium concentration

The calcium ionophore A 23187 (5 µM) rapidly decreased the membrane potential of unstimulated cells (Fig. 3a) and entirely suppressed the FSH-induced membrane hyperpolarization (Fig.

3b). In the steady state, the mean values of membrane potential of unstimulated and FSH-stimulated cells were not significantly different (Table 1). The ionophore effect of FSH-stimulated cells was partly reversed by a 30-min period in the control medium.

Fig. 3. Early effects of A 23187 (5×10^{-6} M) on the membrane potential of (a) unstimulated and (b) FSH-stimulated Sertoli cells. The cells were isolated from 17-day-old rats by Procedure 2 and cultured for 3–7 days before treatment. A 23187 (in stock solution in methanol 2×10^{-3} M) was dissolved in the electrophysiological medium. After the measurement of potential in the control electrophysiological medium ($\bullet$, $\circ$), A 23187 was added and membrane potentials were recorded at different times of exposure (Δ , $\blacktriangle$). After 20 min, A 23187 was washed out and replaced by the control medium. Each point represents the membrane potential of one impaled cell. Data were obtained from 3 cultures.

Table 1. Membrane potentials (mV) of immature rat Sertoli cells in monolayer culture recorded during various experimental procedures

Treatment	Unstimulated cells		FSH-stimulated cells	
	Control	Treatment	Control	Treatment
A 23187 (5 μ M)	-22.7 ± 0.5 (33)	$-16.4 \pm 0.6^*$ (19)	-36.6 ± 0.4 (44)	$-17.5 \pm 0.4^*$ (35)
Heptanol (3.5 mM)	-23.0 ± 0.5 (18)	$-6.5 \pm 0.4^*$ (12)	$-39.7 \pm 0.4^*$ (38)	$-6.4 \pm 0.3^*$ (16)
CCmP (1 μ M)	-21.5 ± 0.3 (13)	-20.4 ± 0.6 (9)	-36.6 ± 0.6 (31)	$-22.8 \pm 0.6^*$ (31)
0 Ca^{2+} + EGTA (1 mM)	-21.4 ± 0.3 (29)	$-20.0 \pm 0.3^*$ (26)	-37.7 ± 0.3 (48)	$-24.5 \pm 0.3^*$ (52)

Values are means $\pm$ s.e.m. of values determined in the steady state, for the no. of cells indicated in parentheses after a 24-h exposure to 2.9 μ g oFSH-S16 (NIADDK).

* $P < 0.01$ compared with control value.

Heptanol (the heptyl alcohol or *n*-heptan-1-ol) is a general anaesthetic which suppresses electrical coupling in several structures (Johnston *et al.*, 1980; Délèze & Hervé, 1983; Bernardini *et al.*, 1984) by an increase of intracellular calcium and an alkalinization of cytosol (Vassort *et al.*, 1986). Applied to Sertoli cells, heptanol (3.5 mM) rapidly induced the depolarization of unstimulated and FSH-stimulated cells (Fig. 4a, b). In the two groups, a similar low membrane potential was recorded ($P > 0.05$; Table 1). The effects of heptanol were entirely reversible (Fig. 4) but the kinetics of the recovery period varied. With unstimulated cells, the membrane potential rapidly returned to the control value (-22.5 ± 0.5 mV; $n = 24$; $P > 0.05$). For the FSH-stimulated cells, the membrane potential rapidly reached the same level (-22.5 ± 0.6 mV; $n = 21$; $P > 0.05$) then slowly rose to the control values for the stimulated cells (Fig. 4; Table 1).

Fig. 4. Early effects of heptanol (3.5 mM) on the membrane potential of (a) unstimulated and (b) FSH-stimulated Sertoli cells. The cells were isolated from 17-day-old rats by Procedure 2 and cultured for 3–7 days before treatment. Heptanol was dissolved in the electrophysiological medium. After the measurement of potential in the control electrophysiological medium (●, ○), heptanol was added and membrane potentials were recorded at different times of exposure (▲, △). After 10 min, heptanol was washed out and replaced by the control medium. Each point represents the membrane potential of one impaled cell. Data were obtained from 3 cultures.

The uncouplers of oxidative phosphorylations reduce the cell-to-cell conductance (Loewenstein, 1981). This reduction is related to a decrease of the intracellular ATP and to a simultaneous increase in the intracellular calcium levels (Politoff *et al.*, 1969; Rose & Rick, 1978; Délèze & Hervé, 1983). Such a drug, carbonyl-cyanid-*m*-chlorophenyl-hydrazone (CCmP) was used to raise the intracellular Ca^{2+} concentration in neurones and in pancreatic β cells (Plant *et al.*, 1983; Rosario

et al., 1985). An exposure to CCmP ($1 \mu\text{M}$) did not significantly decrease the membrane potential of unstimulated cells (Fig. 5a; Table 1), but did abolish the FSH-induced hyperpolarization (Fig. 5b; Table 1). This effect was partly reversed by a 15-min period in the control medium.

Fig. 5. Early effects of CCmP (10^{-6} M) on the membrane potential of (a) unstimulated and (b) FSH-stimulated Sertoli cells. The cells were isolated from 17-day-old rats by Procedure 2 and cultured for 3–7 days before treatment. CCmP was dissolved in electrophysiological medium. After the measurement of potential in the control electrophysiological medium (●, ○), CCmP was added and membrane potentials were recorded at different times of exposure (▲, △). After 20 min, CCmP was washed out and replaced by the control medium. Each point represents the membrane potential of one impaled cell. Data were obtained from 3 cultures.

Decreased extracellular calcium concentration

Exposure to 0 calcium medium did not change the membrane potential of unstimulated cells but slightly decreased from $-36.4 \pm 0.6 \text{ mV}$ ($n = 102$) to $-32.4 \pm 0.6 \text{ mV}$ ($n = 128$) ($P < 0.01$) the FSH-induced hyperpolarization (Joffre & Roche, 1988). A decrease of electrical coupling is observed when *Chironomus* salivary gland cells are exposed to 0 calcium solutions containing EGTA (Loewenstein *et al.*, 1967). Figure 6 shows that an exposure to 0 Ca + EGTA (1 mM) medium (giving about 10^{-9} M calcium) slightly but significantly decreased the membrane potential of unstimulated cells (Table 1) and induced greater depolarization of FSH-stimulated cells ($P < 0.01$).

At the steady state, the membrane potential was higher in the FSH-stimulated than in the unstimulated cells ($P < 0.01$). As soon as the low calcium–EGTA medium was washed out the membrane potential returned to control values.

Effects of ouabain applied to cells

Ouabain (10^{-4} M) does not affect the membrane potential of unstimulated cells, but decreases the membrane potential of FSH-stimulated cells ($P < 0.01$; Joffre & Roche, 1988). When ouabain (10^{-4} M) was applied to unstimulated or FSH-stimulated cells previously depolarized by an uncoupling treatment (e.g. 0 Ca + EGTA), this exposure did not move the membrane potential towards lower values (Fig. 6).

Effects of trypsin treatment and mechanical dispersion

The exposure of *Chironomus* salivary glands to a medium containing trypsin (0.05%) leads to a rapid and irreversible uncoupling of cells (Loewenstein *et al.*, 1967). A similar experimental procedure could not be used in this study, since the early effect of trypsin induced the loosening of the

Fig. 6. Early effects of 0 Ca + EGTA (1 mM) medium on the membrane potential of (a) unstimulated and (b) FSH-stimulated Sertoli cells. The cells were isolated from 17-day-old rats by Procedure 2 and cultured for 3–7 days before treatment. After the measurements of potential in the control electrophysiological medium (●, ○), 0 Ca + EGTA (1 mM) medium was added and membrane potentials were recorded at different times of exposure (▲, △). After 20 min, ouabain (10^{-4} M) was added and membrane potentials were recorded. After 15 min, 0 Ca + EGTA medium containing ouabain was washed out and replaced by the control medium. Each point represents the membrane potential of one impaled cell. Data were obtained from 3 cultures.

monolayer from the bottom of the Petri dish and prevented the use of microelectrode techniques. Therefore, the Sertoli cells were first stimulated by a 24-h exposure to FSH (2.0 µg/ml), then the culture medium was discarded and replaced by 0.5 ml 0 Ca–0 Mg Earle's solution containing trypsin (0.05%). After a 5–10-min period of incubation at 34°C, the monolayers were transferred to a conical tube containing RPMI medium with fetal calf serum (10%) and then centrifuged (70 g, 6 min, 20°C). The pellet was then suspended in Medium RPMI and dispersed by being passed through a syringe needle, as during preparation of the Sertoli cells. The Sertoli cells were then plated by the usual procedure. After a second 3-day culture, the measurement of membrane potential was performed. The data are summarized in Table 2. The FSH-induced hyperpolarization was suppressed and a second exposure to FSH-induced a new hyperpolarization of the cell to values near those of stimulated cells ($P < 0.01$).

Table 2. Effects of a second FSH stimulation on membrane potentials of immature rat Sertoli cells in monolayer culture recorded after a trypsin treatment followed by a mechanical dispersion

Treatment	Membrane potential (mV)
Control (without FSH)	-21.1 ± 0.5 (25)
oFSH (2.9 µg/ml for 24 h)	$-36.6 \pm 0.5^*$ (45)
oFSH (2.9 µg/ml for 24 h) + dispersion	$-19.7 \pm 0.4^{**}$ (36)
oFSH (2.9 µg/ml for 24 h) + dispersion + oFSH (2.9 µg/ml for 24 h)	$-33.8 \pm 0.7^{**}$ (23)

Values are means $\pm$ s.e.m. of values determined in the steady state for the no. of impaled cells indicated in parentheses.

* $P < 0.01$ compared with unstimulated cells.

** $P < 0.01$ compared with cells stimulated only once with FSH.

Discussion

Conventional cultures of Sertoli cells on plastic Petri dishes have been extensively used for studying the structure, function and FSH regulation of cellular metabolism and secretion (Dorrington *et al.*, 1975; Steinberger *et al.*, 1975; Welsh & Wiebe, 1975; Verhoeven *et al.*, 1979; Rich *et al.*, 1983). We decided to use two of them to explore the electrophysiology of Sertoli cells and their FSH stimulation (Joffe & Roche, 1988).

The first experiments were performed on Sertoli cells which were isolated by Procedure 1, but most were carried out on cells isolated by Procedure 2. Procedure 2 gave a more homogeneous monolayer of cells which grew without fetal calf serum, omission of which did not noticeably modify the cell response to FSH (Verhoeven *et al.*, 1980) and enhanced the electrical response to this hormone (Joffe & Roche, 1988).

In spite of the epithelial structure of Sertoli cells in monolayer culture which is different from the cellular organization in testis, our electrophysiological study has yielded results which largely support the radioisotopic and chemical analysis of ionic components of fluid production (for review: Waites & Gladwell, 1982) and the electrophysiological study on the whole seminiferous

tubule (Cuthbert & Wong, 1975). Our study gave maximal values of membrane potential equal to those determined *in situ* through the tubular wall by Cuthbert & Wong (1975).

It showed that ovine FSH caused a dose-dependent hyperpolarization of cell. As the FSH concentration rose from 0 to 2.9 µg/ml, the membrane potential progressively increased with the maximal value equal to -37.0 ± 0.2 mV; $n = 310$) when the cells were prepared by Procedure 2. It is significantly lower when the cells were prepared by the Procedure 1 (-30.5 ± 0.3 mV; $n = 277$; $P < 0.01$). This response appears not to be modified by the culture period (Joffre & Roche, 1988).

Many electrophysiological studies on exocrine glands have shown that treatments which affect the functional state of the junctional complex linking neighbouring cells modify the recordings obtained by the intracellular microelectrode method (Iwatsuki & Petersen, 1978a, b; Loewenstein, 1981). Therefore, with the purpose of examining whether the junctional complex between Sertoli cells may affect the FSH-induced hyperpolarization and to obtain complementary results on the FSH stimulation, Sertoli cells were exposed to treatments known to suppress or to decrease the intercellular electrical coupling in different structures, including salivary gland, myocardium or neurones (Loewenstein *et al.*, 1967; Délèze, 1970; De Mello, 1975; Rose & Loewenstein, 1975, 1976; Johnston *et al.*, 1980; Délèze & Hervé, 1983). All these treatments, which involve an increase in intracellular calcium (A 23187, heptanol, CCmP) or a decrease of extracellular calcium (0 Ca + EGTA) induce a fast and significant depolarization of FSH-stimulated cells to an unstimulated level. All the procedures, with exception of an exposure to CCmP, slightly decrease the membrane potential of unstimulated cells. The effects are either partly (A 23187 and CCmP) or totally (heptanol and 0 Ca + EGTA) reversed by a recovery period in a drug-free medium.

All these results suggest that calcium and/or the degree of coupling between Sertoli cells modify the FSH-induced hyperpolarization. The recorded depolarization may be related to the calcium sensitivity of the Na/K pump (which is inhibited by uncoupling treatments (Fig. 6)) and of the K channels responsible for this hyperpolarization (Joffre & Roche, 1988).

The FSH-induced hyperpolarization did not disappear after a recovery period in a hormone-free medium (Fig. 1), even when the cells were bathed in a pH 3 medium to break down the bound FSH. It was only cancelled after an exposure to trypsin followed by a new mechanical dispersion (Table 2). Such a trypsin treatment reproduced the experimental procedure used for isolating the Sertoli cells from the testes. This may explain the same values of membrane potential measured on unstimulated cells isolated before and after the appearance of the blood-testis barrier (Vitale *et al.*, 1973) and the same response to the FSH stimulation.

Direct communication pathways are present in the seminiferous tubules between neighbouring cells (Johnson, 1970). They are mediated by a junctional complex including tight and gap junctions which were observed *in situ* in the seminiferous tubule (Dym & Fawcett, 1970; Vitale *et al.*, 1973; Gilula *et al.*, 1976) and *in vitro*, either in culture of whole seminiferous tubule (Meyer *et al.*, 1977) or in monolayer cultures of Sertoli cells (Solari & Fritz, 1978; Hadley *et al.*, 1985).

An effect of FSH on the junctional complex was previously determined by an ultrastructural study on a monolayer of Sertoli cells cultured in the same conditions as in our own experiment (Solari & Fritz, 1978). It showed that the Sertoli cells isolated from testes of 10-day-old rats and cultured in medium without FSH did not develop complete junctional complexes. These appeared only when the cells were stimulated by FSH and did not disappear after a recovery period in a hormone-free medium.

Direct evaluations of the electrical coupling between Sertoli cells in culture were made by means of two microelectrodes (Eusebi *et al.*, 1985; unpublished data), and showed that unstimulated cells were effectively coupled. This coupling is decreased by A 23187 (Eusebi *et al.*, 1985). The effects of uncoupling treatments on the membrane potential of cultured Sertoli cells therefore suggest that Sertoli cells are cells in which an electrical uncoupling is associated to a cellular depolarization, as in many cells (Loewenstein, 1981). The results are also consistent with the suggestion that Sertoli cells which should be weakly coupled in an unstimulated state, should be more electrically coupled after an FSH stimulation. Such an increase may be related to an increase in the conductance and/or

to an increase in the number of gap junctions between neighbouring cells (Loewenstein, 1981). The effects of dibutyryl cAMP (Joffre & Roche, 1988) suggest that this process is a cAMP-dependent mechanism as in many structures (Loewenstein, 1981; Hertzberg *et al.*, 1981; Spray & Bennett, 1985).

In the present study, the Sertoli cells grew in a conventional culture system, on a plastic Petri dish. They present little resemblance to the highly polarized cells *in vivo*. They have little cytoplasm and cell-cell junctions are occasionally located at the apex (Solari & Fritz, 1978; Hadley *et al.*, 1985). In these models high doses of FSH were generally used to stimulate the cells (Steinberger *et al.*, 1978; Verhoeven *et al.*, 1979). Similar high levels of FSH were used here to induce a membrane potential equal to those measured *in vivo*.

Attempts have been made to improve the morphology of Sertoli cells and prolong their secretory activity in culture. These include the use of serum-free and hormone-supplemented media, various reconstituted basal membranes and cell culture chambers with two compartments (Rich *et al.*, 1983; Hadley *et al.*, 1985, 1987; Djakiew *et al.*, 1986; Janecki & Steinberger, 1987). In these systems, Sertoli cells form a polarized monolayer with characteristic basally located tight junctions and they maintain germ cells in the basal part of the monolayer. Their secretory process is enhanced and they are more sensitive to FSH. Attempts are now being made to carry out these electrophysiological studies of FSH on such a model and to determine the effects of insulin and transferin on the FSH-induced hyperpolarization.

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