J. Membrane Biol. 169, 167–174 (1999)

The Journal of
Membrane
Biology



# Inhibition by cAMP of Calcium-Activated Chloride Currents in Cultured Sertoli Cells from Immature Testis

**N. Lalevée, M. Joffre**

Laboratoire de Physiologie des Régulations Cellulaires, UMR 6558, UFR Sciences, 40 Avenue du Recteur Pineau 86022-Poitiers-Cedex, France

Received: 30 November 1998/Revised: 2 March 1999

**Abstract.** We have characterized a $Ca^{2+}$-dependent $Cl^-$ current ($Cl_{Ca}$) in cultured Sertoli cells from immature rat testis by using the whole cell recording patch-clamp technique. Cells dialyzed with pipette solutions containing 3 mM adenoside-triphosphate (ATP) and 1 μM free $Ca^{2+}$, exhibited outward currents which were inhibited by 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) and anthracene-9-carboxylic acid (9-AC) but insensitive to tetraethylammonium (TEA). Dialysis of cells with pipette solutions containing less than 1 nM free $Ca^{2+}$ strongly reduced the currents indicating that they were $Ca^{2+}$ dependent. With cells dialyzed with $Cs^+$ glutamate-rich pipette solutions containing 0.2 mM EGTA, 10 μM ionomycin induced outward currents having properties of $Ca^{2+}$-activated $Cl^-$ currents.

With ATP-free pipette solution, the magnitude of currents was not modified suggesting the direct control by $Ca^{2+}$. By contrast, addition of 0.1 mM cAMP in the pipette solution or the superfusion of cells by a permeant analogue of cAMP strongly reduced the currents. These results may suggest that $Cl_{Ca}$ is inhibited by cAMP-dependent protein kinase.

Finally, our results do not agree with the model of primary fluid secretion by exocrine cells, but are in agreement with a hyperpolarizing effect of cAMP in primary culture of Sertoli cells and the release of a low $Cl^-$ and bicarbonate-rich primary fluid by these cells.

**Key words:** Sertoli cells — Cytosolic calcium — cAMP — $Ca^+$ activated — Chloride current — Chloride conductance

## Introduction

Sertoli cells from the mammalian testis are responsible for the formation and the secretion of a tubular fluid. It allows the proliferation and the differentiation of germ cells, and the transport of spermatozoa into the epididymis. Micropunctures and efferent duct ligatures have been extensively used to collect fluid samples from the seminiferous tubules. This intratubular fluid is a composite of compounds transferred across the Sertoli cells, the primary fluid, that forms an admixture with locally secreted and absorbed products from the germinal cells (for review *see* Waites & Gladwell, 1982; Hinton & Setchell, 1993). However, the composition of the fluid secreted by Sertoli cells is not perfectly determined.

Electrophysiological techniques have also been used to investigate the membrane processes underlying the fluid secretion by Sertoli cells. The intracellular microelectrodes have been applied to whole seminiferous tubules (Cuthbert & Wong, 1970; for review *see* Waites & Gladwell, 1982), to immature Sertoli cells (Joffre & Roche, 1988; Roche & Joffre, 1989; Wassermann et al., 1992*a*, *b*; Loss et al. 1998) and TM4 Sertoli cell line (Jungwirth et al., 1997) in culture. More recently, the whole cell configuration of the patch-clamp technique has been applied to Sertoli cells (Lalevée, Pluciennik & Joffre, 1997; Boockfor et al., 1998). We recently characterized $Ca^{2+}$ currents in Sertoli cells having the properties of T type $Ca^{2+}$ channels (Lalevée, Pluciennik & Joffre, 1997). The mechanisms by which the primary fluid is secreted as well as its control by FSH are still puzzled out. In the present study, we have investigated these processes using the patch-clamp technique applied to immature Sertoli cells in primary culture. We have identified a calcium-activated chloride current that is inhibited by cAMP in the presence of ATP.

## Materials and Methods

### Sertoli Cell Preparation and Culture

Sertoli cells were isolated as previously described (Lalevée, Pluciennik & Joffre, 1997). The testes of immature (12–14 days old) Wistar rats

*Correspondence to:* M. Joffre

were submitted to a three-step enzymatic digestion by collagenase (0.25 mg/ml, Worthington Biochemical, Freehold, N.J., 291 U/mg), pancreatin (0.5 mg/ml, grade VI, Sigma, Chemical, St. Louis, MO), then by trypsine-EDTA (5 mg/ml, Life Technologies, Paisley, Scotland) in a $Ca^{2+}$ and $Mg^{2+}$-free modified Earle's solution. This medium contained (in mM): 116.3 NaCl, 5.4 KCl, 0.9 $NaH_2PO_4$, and 5.5 glucose, supplemented with 53.5 mannitol, 20 HEPES, and streptomycin sulphate (100 μM) and penicillin G (100 IU/ml, Sigma). The pH was adjusted to 7.4. Cells were then resuspended in the presence of trypsin inhibitor (0.1 mg/ml, Soybean-type 1S, Sigma). The cell density was measured with a haematocytometer, and their viability was determined by trypan blue exclusion. The preparations contained more than 99% living cells in which there were more than 95% Sertoli cells identified by light microscopy. Final cell preparations were plated at 55,000 cells/$cm^2$ in 35 mm plastic petri dishes (Nunclon, Nunc, Roskilde, Denmark). The culture medium (RPMI 1640, Life Technologies) was supplemented with L-glutamine (2 mM), transferrin (0.005 mg/ml), insulin (0.01 mg/ml), BSA (1 mg/ml), HEPES (10 mM), sodium bicarbonate (20 mM), streptomycin sulphate (100 μM) and penicillin G (100 IU/ml) (all from Sigma). The culture dishes were maintained at 34°C in a humidified atmosphere of 95% air/5% $CO_2$ for 2 to 5 days. The medium was changed after 48 hr, then every 2 days.

### Electrophysiology

Membrane currents were obtained by the whole-cell configuration of the patch-clamp technique as described by Marty & Neher (1983, 1995). Recording pipettes with resistances ranged from 2 to 4 MΩ were pulled from borosilicate glass capillary tubing (GC150-TF10, Clark Electromedical, UK) using a two-step vertical electrode puller (type PP-82, Narishige, Japan). They were fire-polished and connected to the head-stage of the patch-clamp amplifier (EPC-7, List Medical, Darmstadt, Germany) through an Ag/AgCl pellet. Seal resistances ranged from 3 to 30 GΩ.

The voltage-clamp programs and data collection were performed with a microcomputer (PS1 486DX) equipped with an analog/digital-digital/analog conversion board (TM-40, Teckmar) and a specific software (pClamp 5.5.1., Axon Instruments, Foster City, CA). Cell currents were lowpass filtered at 3.3 kHz, digitized online with the pClamp software after filtering (cutoff frequency 0.3 kHz) and stored on disk. Pipette capacitances were electronically compensated in cell-attached mode. Membrane capacitances and series resistances were measured in the whole-cell mode by fitting capacitance currents with a first order exponential, and by integrating the surface of capacitance current. They were not compensated. Average series resistances and membrane capacitances were, respectively, 11.7 ± 0.3 MΩ ($n = 162$) and 13.3 ± 0.4 pF ($n = 194$).

### Solutions

At the beginning of each experiment, the petri dish was rinsed several times with the standard extracellular solution to remove cells which were not attached. Test solutions were applied rapidly by directing a streamline flow from the opening of a plastic capillary with an internal diameter of 250 μm positioned in the bath near the investigated cell. All recordings were performed at room temperature (20–23°C).

The standard extracellular solution consisted of (mM): 161 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 5 HEPES-NaOH buffer, pH = 7.4. Two intrapipette solutions were used with different EGTA-calcium buffers to hold the intracellular free calcium concentration at 1 nM [A] and 1 mM [B]. They consisted of (in mM) [A]: 1 NaCl, 57 KCl, 88 KGlutamate, 3 $MgCl_2$, 0.2 EGTA, 10 HEPES/KOH buffer, pH = 7.2, and [B] (in mM): 1 NaCl, 39 KCl, 81 KGlutamate, 3 $MgCl_2$, 10 EGTA, 9.1 $CaCl_2$, 10 HEPES/KOH buffer, pH = 7.2. The equilibrium potentials for the ions were (mV ± 1 mV): $E_{K^+} = -85$, $E_{Na^+} = +129$, $E_{Cl^-} = -25$, $E_{Cat^+}$: [A] = +3 and [B] = +8. Depending on the experiments, 3 mM ATP and/or 0.1 mM cAMP were added to the pipette solutions as indicated in Results.

The effects of 10 mM TEA (tetraethylammonium), a $K^+$ current inhibitor, of 1 mM DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid) and 1 mM 9-AC (anthracene-9-carboxylic acid), two well-known $Cl^-$ current inhibitors, have been investigated on the currents. These drugs were dissolved in DMSO as 200 mM stock solution then in the extracellular solution with a final DMSO concentration of 0.5%.

The chloride selectivity of the current was investigated when $Cs^+$ substituted $K^+$ and $Na^+$ pipette and bath solutions, respectively. The extracellular solution consisted of (mM): 160 CsCl, 2 $CaCl_2$, 2 $MgCl_2$, 5 HEPES/CsOH buffer, pH 7.4. The intrapipette solution was (mM): 10 CsCl, 130 KGlutamate, 3 $MgCl_2$, 0.5 EGTA, 10 HEPES/KOH buffer, pH 7.2. In these conditions, the effect of 10 μM ionomycin on $Ca^{2+}$-activated chloride currents was investigated. Ionomycin was dissolved in DMSO as 1 mM stock solution then in the extracellular solution with a final DMSO concentration of 1%. To confirm the presence of chloride currents, $E_{Cl}$ was moved from −25 to +40 mV by replacing NaCl by equimolar NaGlutamate. Currents in control experiments were not altered by DMSO (1%). Junction potentials developed at the tip of pipettes in all the described conditions has been corrected according to the method of Fenwick, Marty, & Neher (1982).

## Results

### Elevation of $[Ca^{2+}]_i$ Activates a $Cl^-$ Current

Sertoli cells were first bathed in the standard extracellular solution and whole cell currents were recorded in $KCl_{in}/NaCl_{out}$ solutions, when depolarizations were applied from −50 to +70 mV in increments of 20 mV from a holding potential of −50 mV. Figure 1*A* (filled squares) illustrated current traces obtained with an intracellular calcium concentration less than 1 nM (pCa > 9) and with 3 mM ATP. Depolarizations elicited small, time-independent currents. The current-voltage curve resulting from the mean current measured at the end of pulses ($n = 8$), was quite linear with an amplitude averaging 3.7 ± 0.7 pA/pF at +70 mV (Fig. 1*D*, filled squares). This current reversed near −20 mV. Because of the small amplitude, the nature of this current was not further analyzed.

In the presence of ATP, when the intracellular calcium concentration was raised to 1 μM (pCa = 6), the current amplitude was increased, and for potentials over +10 mV, a time-dependent activation was observed (Fig. 1*B*, filled circles). The averaged current-voltage curve, resulting from the mean current measured at the end of pulses ($n = 12$), showed an outward rectification for potentials over −10 mV. The current amplitude averaged 13.2 ± 2.2 pA/pF at +70 mV ($P < 0.002$). The reversal potential was not modified (Fig. 1*D*). When ATP was omitted from the pCa = 6 intracellular solution, current amplitudes were found that were similar to those ob-

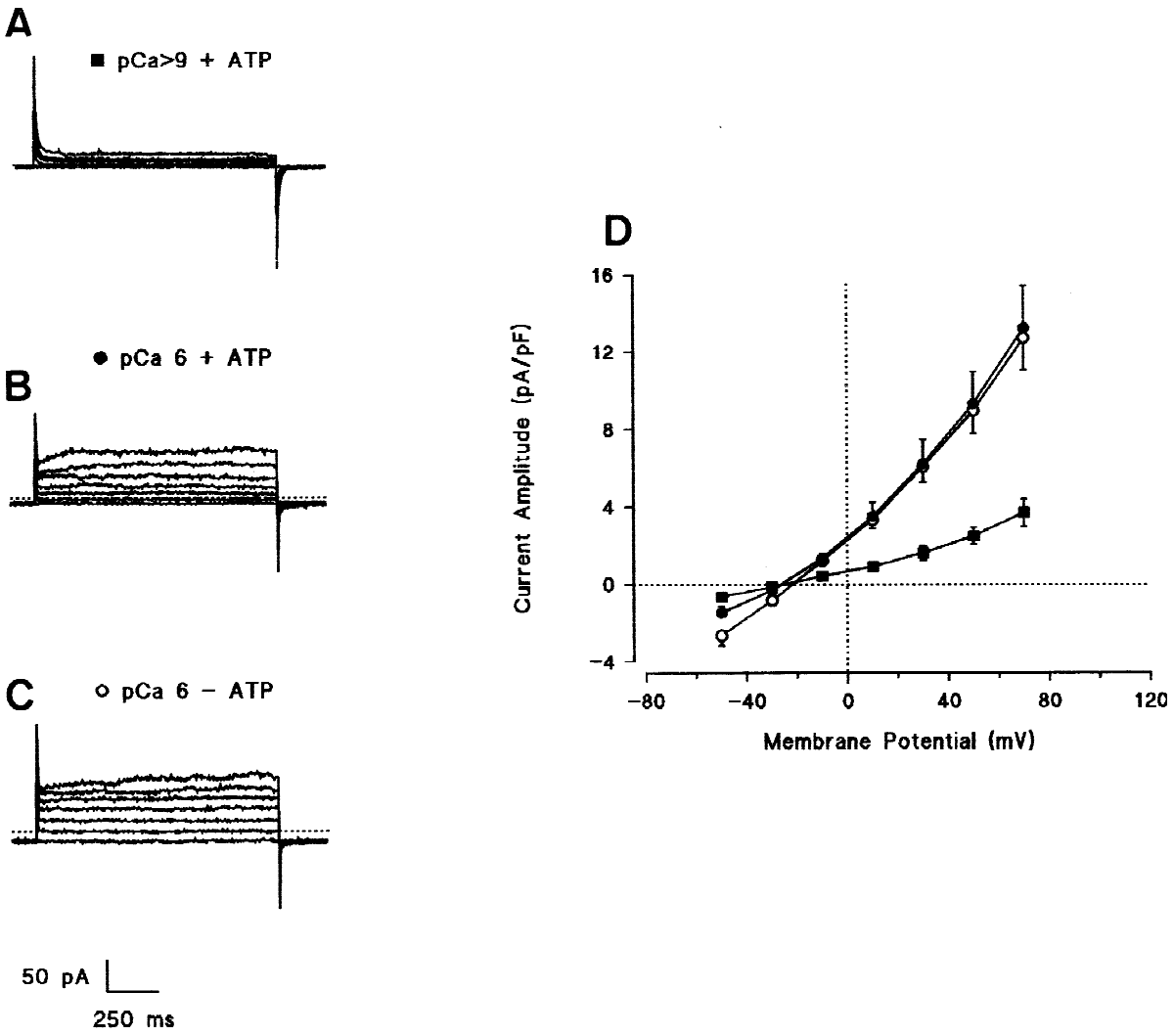


**Fig. 1.** Whole cell currents recorded from cells dialyzed with internal solutions containing 3 mM ATP and less than 1 nM $Ca^{2+}$ (*A*), 3 mM ATP and 1 μM $Ca^{2+}$ (*B*) and 1 μM $Ca^{2+}$ without ATP (*C*). Depolarizations were applied from −50 to +70 mV in 20 mV steps. The dashed lines show zero-current levels. (*D*) Corresponding current-voltage relationships of steady-state currents recorded in *A*, *B* and *C*. Mean values ± SEM for $n = 8$ (*A*), $n = 12$ (*B*), and $n = 12$ (*C*).

served with ATP (Fig. 1*C*, open circles), and the current amplitude at +70 mV was not significantly different 12.7 ± 1.6 pA/pF ($n = 12$) (Fig. 1*D*, open circles). A small inward current was also recorded at −80 mV which could result from the activation of a ATP-inhibited $K^+$ current (*not shown*).

To further characterize the whole cell currents induced by $[Ca^{2+}]_i$, we tested TEA, DIDS and 9-AC, respectively, a $K^+$ and two $Cl^-$ channel blockers. Figure 2 illustrated current traces obtained with an intracellular calcium concentration of pCa 6 and with 3 mM ATP. Figure 2*A* and *C*, shows that the addition of 10 mM TEA did not modify the outward whole cell current ($n = 3$). In contrast, 1 mM DIDS (Fig. 2*A* and *C*; $n = 3$) and 1 mM 9-AC (Fig. 2*B* and *D*; $n = 3$) strongly inhibit the outward current.

To study the anion selectivity of the $[Ca^{2+}]_i$-induced current, we performed experiments in two conditions (Figs. 3 and 4). Experiments yielding the data illustrated in Fig. 3 ($n = 3$) were performed in chloride solutions with an $E_{Cl^-} = -25$ mV then +40 mV. In such conditions the $E_{rev}$ was shifted from −20 to −2 mV indicating that the $[Ca^{2+}]_i$-activated current was essentially chloride selective. We then performed experiments with the $Ca^{2+}$ ionophore ionomycin to increase $[Ca^{2+}]_i$ in conditions where the potassium was suppressed from the solutions by using Cs-Chloride and Cs-Glutamate ($E_{Cl} = -2$ mV) ($n = 4$). Figure 4 shows three current traces for a depolarization of +74 mV recorded before (*a*), during (*b*) and after (*c*) the cell exposure to 10 μM ionomycin. In control conditions, the depolarization did not activate any current. In contrast, elevation of $[Ca^{2+}]_i$ by the ap-

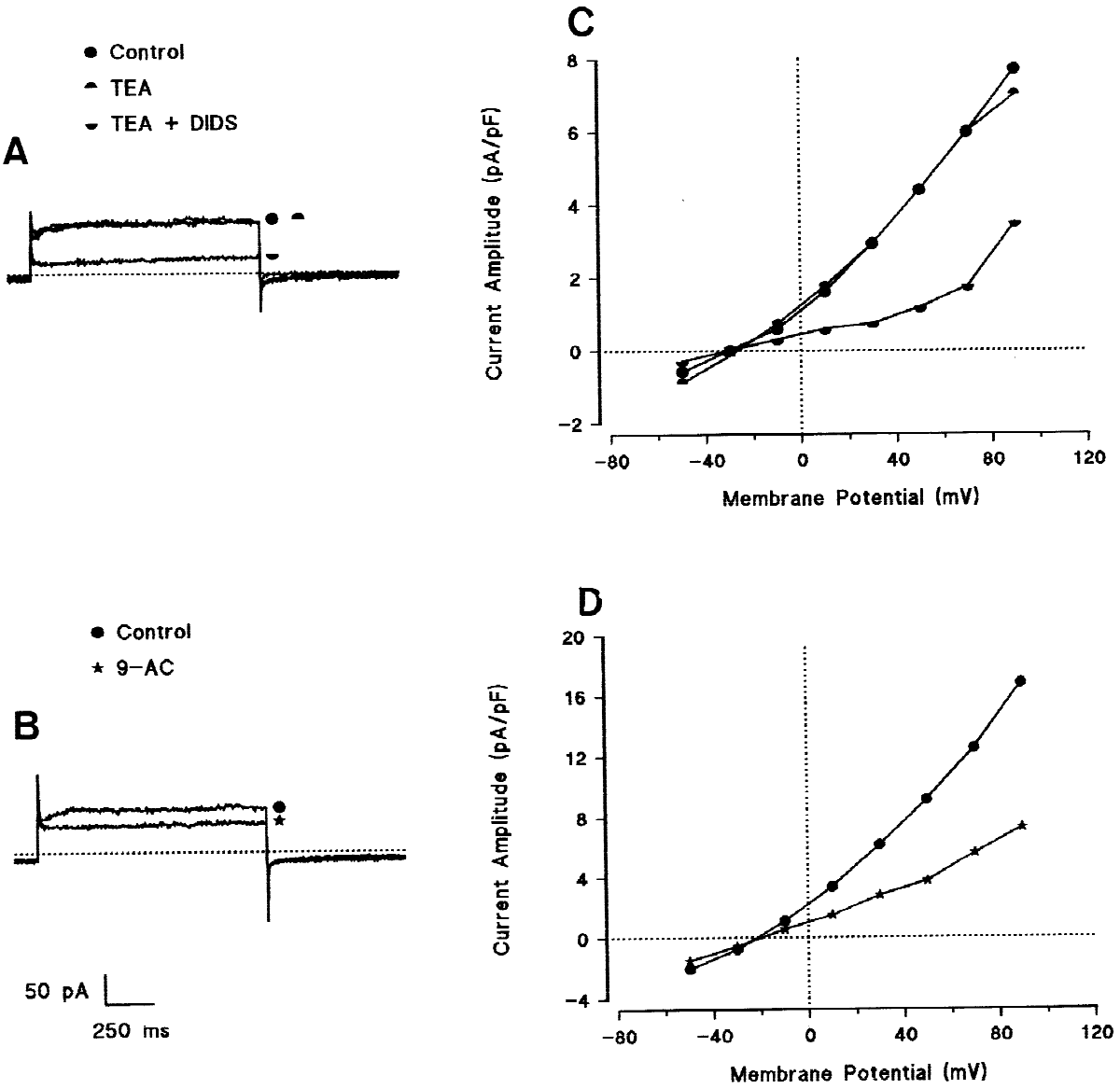


**Fig. 2.** Effects of $K^+$ and $Cl^-$ inhibitors. Cells were dialyzed with internal solutions containing 3 mM ATP and 1 μM $Ca^{2+}$. Current traces were obtained in two different cells, in response to voltage steps from −50 to + 70 mV. (*A*) In control conditions, after the perifusion of 10 mM TEA, then 10 mM TEA + 1 mM DIDS. (*B*) In control conditions and after perifusion of 1 mM 9-AC. (*C*) Current-voltage relationships of steady-state currents recorded from experiment illustrated in *A* and (*D*) from experiment illustrated in *B*.

plication of ionophore activated a large outward current at +74 mV and a much smaller inward current at −46 mV. This current showed the well-known "on" relaxation when activated by depolarization, and the "off" relaxation, when deactivated on return to −46 mV.

These observations were consistent with the direct activation by $[Ca^{2+}]_i$ of a $Cl^-$ current in Sertoli cells, which did not involve any channel phosphorylation. Moreover, the pharmacological sensitivity of the current highly suggested that there was no $[Ca^{2+}]_i$-activated $K^+$ current in Sertoli cells.

### cAMP-Dependent Inhibition of the $Ca^{2+}$-Activated $Cl^-$ Current

$Ca^{2+}$-dependent $Cl^-$ current was, first, fully activated by depolarizations applied to cells dialyzed with the pCa = 6 intracellular solution, in the presence of 3 mM ATP. The resulting currents showed a time-dependent activation for depolarizations over −10 mV (Fig. 5*A*, filled circles) and a time-dependent deactivation for hyperpolarizing pulses of potential.

The amplitude of the currents and the outward rec-

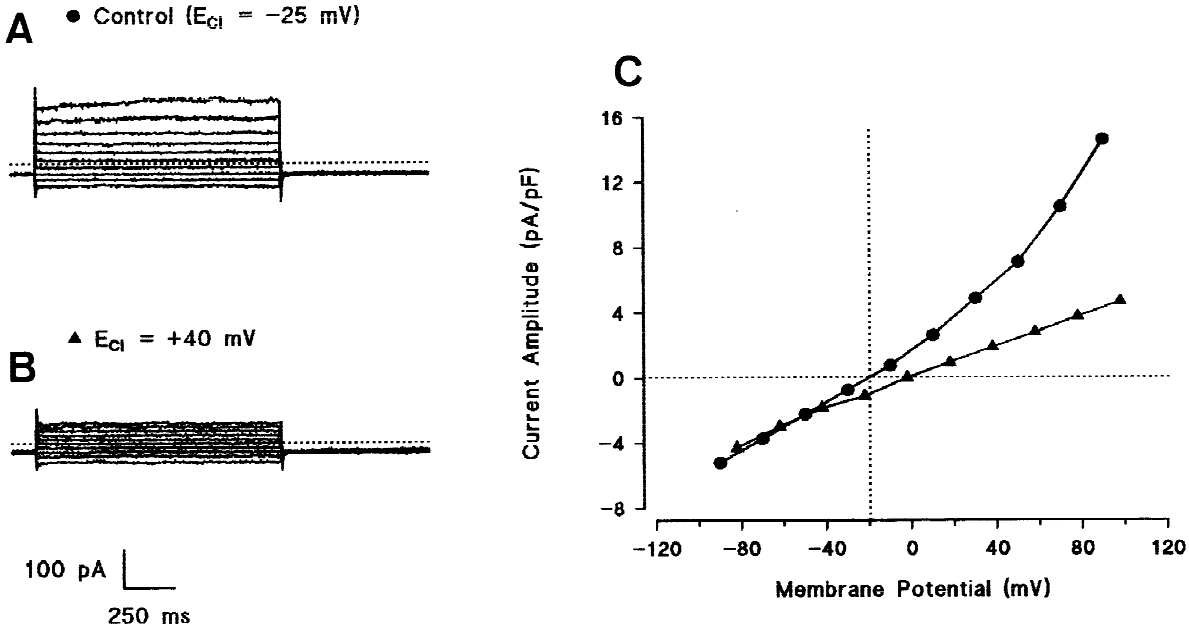


**Fig. 3.** Effects of a shift of $E_{Cl}$. Pulses were applied from −50 mV to potentials varying from −90 to +90 mV when the cell was bathed in control conditions ($E_{Cl}$ = −25 mV) (*A*) then when $E_{Cl}$ was shifted to +40 mV (*B*). (*C*) Current-voltage relationships of steady-state currents recorded from experiments illustrated in *A* and *B*.

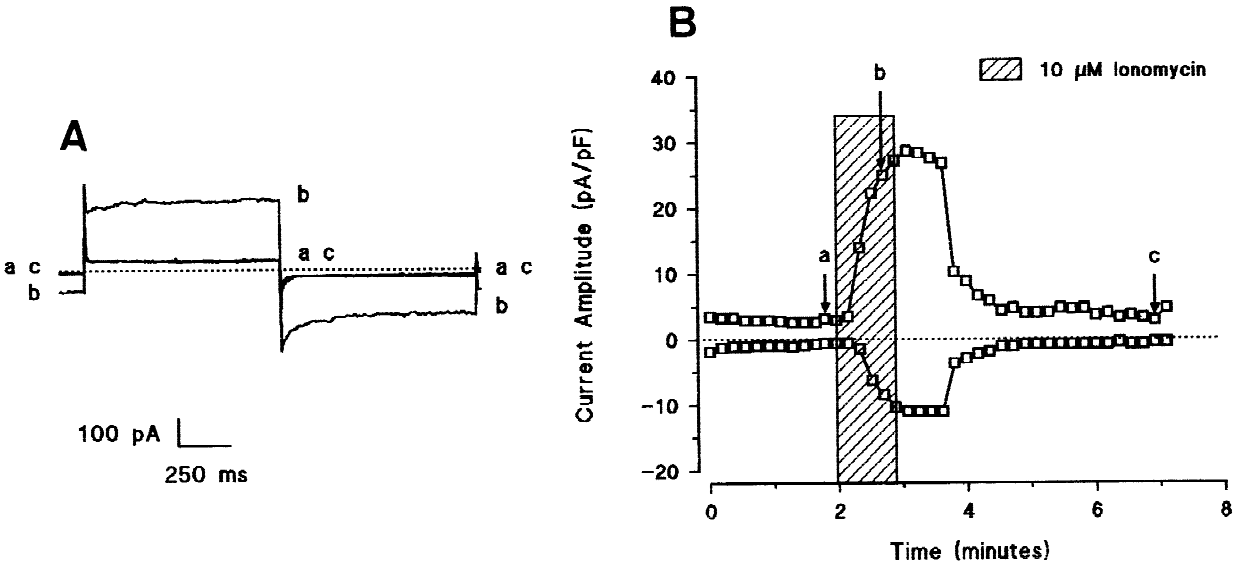


**Fig. 4.** Effects of ionomycin in $Cs^+$ solution conditions. (*A*) Membrane current traces were recorded during voltage steps from −46 to +74 mV. Cell was bathed in control solution then perifused with 10 μM ionomycin and washed with the control solution. (*B*) Whole cell current recorded at −46 and +74 mV when the cell was bathed in control solution then perifused by 10 μM ionomycin (hatched bar) and during the wash.

tification of the current-voltage relationships were strongly reduced when cells were perifused with a solution containing 1 mM CPT-cAMP, a permeant analogue of cAMP (Fig. 5*B* and *C*, open triangles). In these conditions, the current amplitudes were reduced from 20.1 ± 4.3 pA/pF to 10.9 ± 2.7 pA/pF ($n = 6$; $P < 0.02$), while the reversal potential was not modified by CPT-cAMP.

Figure 6 illustrates three series of experiments indicating that the internal cAMP in the presence of ATP totally suppressed the activating effect of $[Ca^{2+}]_i$ on $Cl^-$ current. These current-voltage relationships resulted from currents which were recorded for depolarizations between −50 and +70 mV applied from a holding potential of −50 mV. There was no significant difference be-

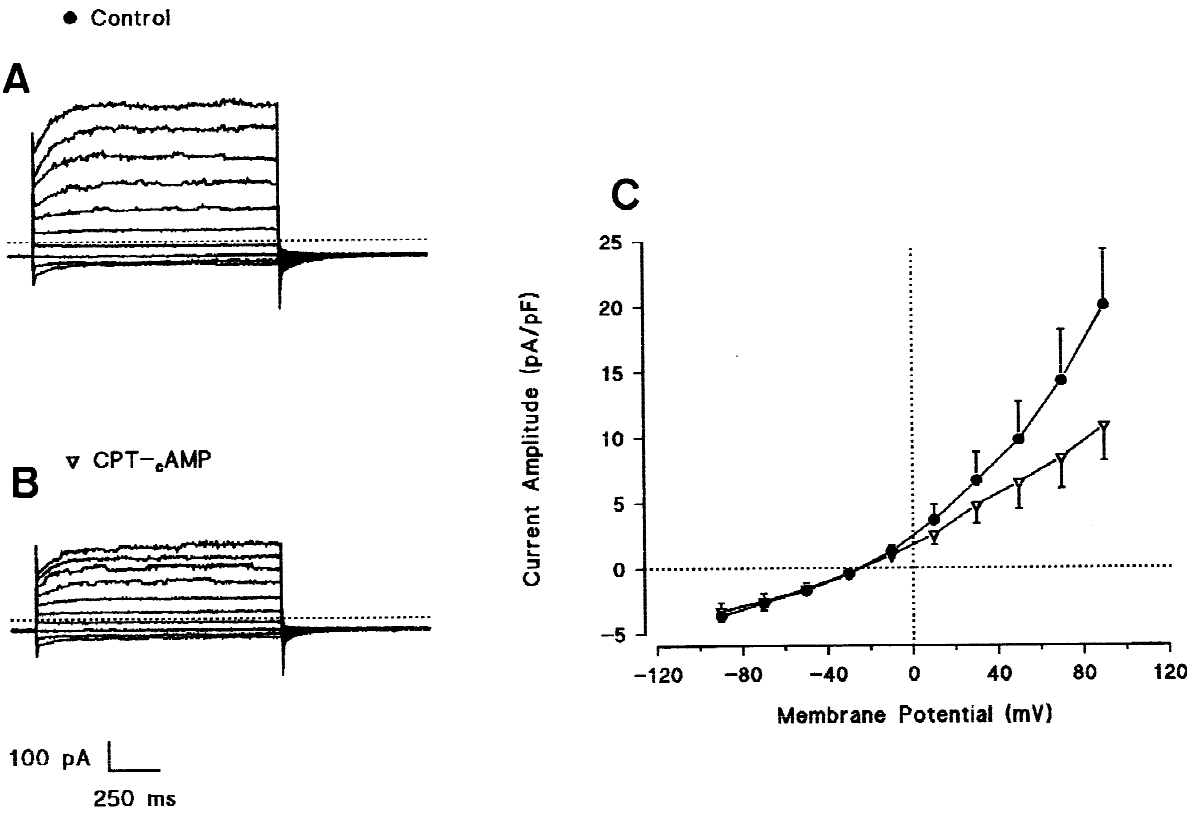


**Fig. 5.** Inhibition of $Cl^-$ currents by cAMP. Pulses were applied from −50 mV to potentials varying from −90 to +90 mV when the cell was bathed in control conditions (*A*) and when 1 mM CPT-cAMP was perifused (*B*). (*C*) Current-voltage relationships of steady-state currents recorded from experiments illustrated in *A* and *B*. Mean values ± SEM for 6 cells.

tween the currents recorded at pCa > 9 with 3 mM ATP, in the presence ($n = 6$; open triangles) or in the absence ($n = 8$, filled squares) of 0.1 mM cAMP in the intracellular solution. When 0.1 mM cAMP in the presence of ATP was added to the pCa 6 intracellular solution, the currents were not significantly different from those recorded at pCa > 9 ($n = 3$; filled triangles).

## Discussion

The Sertoli cells from the mammalian testis are recognized as the cells responsible for the formation and secretion of a specialized fluid that provides a microenvironment for germ cells (Hinton & Setchell, 1993). The aim of this study was to characterize ionic currents in immature Sertoli cells and to associate them with the cellular secretion of primary fluid. In cultured Sertoli cells, we show that the internal $Ca^{2+}$ activates an outward rectifying current. This current reverses to the $Cl^-$ equilibrium potential and is inhibited by the classical $Cl^-$ channel inhibitors, DIDS and 9-AC. Besides, this current is insensitive to TEA, a strong inhibitor of $Ca^{2+}$-

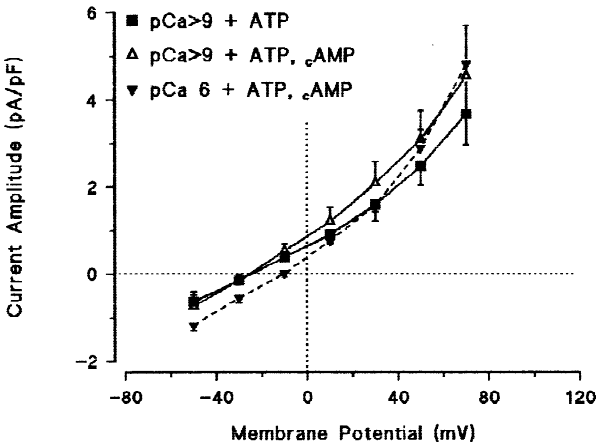


**Fig. 6.** Antagonistic effects of intracellular cAMP and $Ca^{2+}$ on Ca-activated Cl current. Current-voltage relationships of steady-state currents recorded for the three conditions when cells were depolarized from −50 to +70 mV in increments of 20 mV. Internal solutions contained less than 1 nM $Ca^{2+}$ and 3 mM ATP (■), less than 1 nM $Ca^{2+}$, 3 mM ATP and 0,1 mM cAMP (△) and 1 mM $Ca^{2+}$, 3 mM ATP and 0,1 mM cAMP (▼). Mean values ± SEM for $n = 8$ (■), $n = 6$ (△) and $n = 3$ (▼).

dependent $K^+$ current. We conclude that the internal $Ca^{2+}$ activates a $Ca^{2+}$-dependent $Cl^-$ current.

$Ca^{2+}$-dependent $Cl^-$ currents have been shown in various epithelial and nonepithelial cells (Marty, Tan & Trautmann, 1984; Frizzell et al., 1986; Duchatelle & Joffre, 1987; Cliff & Frizzell, 1990; Watanabe, Yumoto & Ochi, 1994; Arreola, Melvin & Begenisich, 1998). Depending on cell types, $Ca^{2+}$ may activate the $Cl^-$ conductance either by interacting directly with channels or by a $Ca^{2+}$/Calmoduline-dependent phosphorylation of $Cl^-$ channels occurring in the presence of ATP. We show that, in the absence of ATP in the pipette, the activation of the $Ca^{2+}$ dependent $Cl^-$ current in immature Sertoli cells may occur, indicating that $Ca^{2+}$ seems to interact directly with $Cl^-$ channels.

In various epithelial cells, the effects of $Ca^{2+}$ and cAMP on chloride currents are additive (Cliff & Frizzell, 1990; Anderson & Welsh, 1991). This occurs by two types of chloride conductance which have been well characterized, the $Ca^{2+}$-dependent $Cl^-$ channels and the ATP and cAMP-dependent $Cl^-$ channels, called CFTR (Cystic Fibrosis Transmembrane conductance Regulator). CFTR is activated by ATP and phosphorylation by the cAMP-dependent Kinase (Kinase A) (Cliff & Frizzell, 1990; Anderson & Welsh, 1991). $Cl_{cAMP}$ current differs from the $Ca_{Ca}$ current in that it is time-independent, shows a linear current-voltage relationship and is insensitive to the stilbene derivatives, DIDS and SITS. The magnitude of $Cl_{cAMP}$ currents is very low, that imposes to record the current in the absence of internal $K^+$ and external $Na^+$. Herein, the experimental procedure did not allow us to record such currents that have been observed in Sertoli cells from adult Sprague Dawley rats (>70 days) (Boockfor et al., 1998) and in TM 4 Sertoli cell line (Jungwirth et al., 1997). In the present study, we show that cAMP in the presence of ATP in the internal medium strongly inhibits the $Ca^{2+}$-mediated activation of $Cl^-$ channels so that the effect of 1 $\mu$M $[Ca^{2+}]_i$ is abolished.

### Functional Significance for Secretion

In the currently accepted model for fluid and electrolyte secretion of acinar cells from epithelium, a rate limiting step in secretion is the $Cl^-$ conductances in the apical membrane. In this type of exocrine cells, two messengers acting by two different pathways stimulate the secretion. In many instances, the effects of calcium are additive or synergistic to those of cAMP. Results as such are not consistent with this accepted model. In Sertoli cells, $[Ca^{2+}]_i$ does not simultaneously activate a $K^+$ conductance, and the effects of cAMP are antagonistic to those of $[Ca^{2+}]_i$. However, the hyperpolarization of Sertoli cells in primary culture (Joffre & Roche, 1988) and the release of a low $Cl^-$ and bicarbonate-rich primary fluid (Tuck et al., 1970) may originate from the blockade of the $Ca^{2+}$-dependent $Cl^-$ current by phosphorylation.

Nathalie Lalevée was a fellow of the Ministère Français de la Recherche et de l'Enseignement Supérieur. Present address: IGH, UPR 1142 CNRS, 141 rue de la Cardoille, 34 396 Montpellier, cedex 5, France. The authors thank Frédérik Becq and Christian Rogier for helpful comments on the manuscript.

## References

Anderson, M.P., Welsh, M.J. 1991. Calcium and cAMP activate different chloride channels in the apical membrane of normal and cystic fibrosis epithelia. *Proc. Natl. Acad. Sci. USA* **88:**6003–6007

Arreola, J., Melvin, J.E., Begenisich, T. 1998. Difference in regulation of $Ca^{2+}$-activated conductances $Cl^-$ channels in colonic and parotid secretory cells. *Am. J. Physiol.* **274:**C161–C166

Boockfor, F.R., Morris, R.A., DeSimone, D.C., Hunt, D., Walsh, K.B. 1998. Sertoli cell expression of the cystic fibrosis transmembrane conductance regulator. *Am. J. Physiol.* **274:**C922–C930

Cliff, W.H., Frizzell, R.A. 1990. Separate $Cl^-$ conductances activated by cAMP and $Ca^{2+}$ in $Cl^-$ secreting epithelial cells. *Proc. Natl. Acad. Sci. USA* **87:**4956–4960

Cuthbert, A.W., Wong, P.Y.D. 1970. Intracellular potentials in cells of the seminiferous tubules of rats. *J. Physiol.* **248:**173–191

Duchatelle, P., Joffre, M. 1987. Ca-dependent chloride and potassium currents in rat Leydig cells. *FEBS Lett.* **217:**11–15

Fenwick, E.M., Marty, A., Neher, E. 1982. A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *J. Physiol.* **331:**577–597

Frizzell, R.A., Halm, D.R., Rechkemmer, G., Shoemaker, R.L. 1986. Chloride channel regulation in secretory epithelia. *Fed. Proc.* **45:**2727–2731

Hinton, B.T., Setchell, B.P. 1993. Fluid secretion and movement. *In:* The Sertoli Cell. Vol. 12. pp. 249–267, Cache River Press,

Joffre, M., Roche, A. 1988. Follicle-stimulating hormone induces hyperpolarization of immature rat Sertoli cells in monolayer culture. *J. Physiol.* **400:**481–499

Jungwirth, A., Weiger, T., Singh, S.K., Paulmichl, M., Frick, J. 1997. Follicle-stimulating hormone activates a cAMP-dependent chloride conductance in TM4 Sertoli cells. *Biochem. Bioph. Res. Com.* **233:**203–206

Lalevée, N., Pluciennik, F., Joffre, M. 1997. Voltage-dependent calcium current with properties of T-type current in Sertoli cells from immature rat testis in primary culture. *Biol. Reprod.* **56:**680–687

Loss, E.S., Barreto, K.P., Leite, L., Wassermann, G.F. 1998. Comparative study of the actions of isoproterenol and retinol on amino acid accumulation, $^{45}Ca$ uptake and membrane potential in Sertoli cells. *Med. Sc. Res.* **26:**195–199

Marty, A., Neher, E. 1995. Tight seal whole cell recording. *In:* Single-Channel Recording. Second Edition, B. Sackmann and E. Neher, Editors. pp. 31–52, Plenum Press, NY

Marty, A., Tan, Y.P., Trautmann, A. 1984. Three types of calcium-dependent channels in rat lacrimal gland. *J. Physiol.* **357:**293–325

Roche, A., Joffre, M. 1989. Effect of uncoupling treatments on FSH-induced hyperpolarization of immature rat Sertoli cells from Sertoli cell-enriched cultures. *J. Reprod. Fert.* **85:**343–354

Tuck, R.R., Setchell, B.P., Waites, G.M.H., Young, J.A. 1970. The composition of fluid collected by micropuncture and catheterization from the seminiferous tubules and rete testis of rats. *Pfluegers Arch.* **318:**225–243

Waites, G.M.H., Gladwell, R.T. 1982. Physiological significance of fluid secretion in the testis and blood testis barrier. *Physiol. Rev.* **62:**624–671

Wassermann, G.F., Monti Bloch, L., Grillo, M.L., Silva, F.R.M.B., Loss, E.S., McConnell, L.L. 1992*a*. Biochemical factors involved in the FSH action on amino acid transport in immature rat testes. *Horm. Metab. Res.* **24:**276–279

Wassermann, G.F., Monti Bloch, L., Grillo, M.L., Silva, F.R.M.B., Loss, E.S., McConnell, L.L. 1992*b*. Electrophysiological changes of Sertoli cells produced by the acute administration of amino acid and FSH. *Horm. Metab. Res.* **24:**326–328

Watanabe, M., Yumoto, K., Ochi, R. 1994. Indirect activation by internal calcium in endothelial cells. *Jap. J. Physiol.* **44:**S233–S236