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Ca-dependent-chloride and potassium currents in rat Leydig cells

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Voltage-clamped membrane currents have been investigated from whole-cell patch-clamp recordings performed on single Leydig cells isolated from the adult rat testis. Two outward membrane currents were evoked by depolarizing voltage steps. A potassium current was recorded in cells dialyzed with low (10^{-9} – 10^{-8} M) calcium media. This current was decreased by TEA (10 mM). A chloride current was recorded in cells dialyzed with high ($10^{-7} - 10^{-6}$ M) calcium media. This current was decreased by an external exposure to glutamate. Comparison of the currents at low and high internal calcium concentrations suggests that an increase of the intracellular calcium activates a chloride current.

Whole-cell patch clamp; K^+ current; Cl^- current; Ca^{2+} sensitivity; (Leydig cell)

1. INTRODUCTION

The gonadotropins (LH, luteinizing hormone; hCG, human chorionic gonadotropin) are known to stimulate steroidogenesis in rat Leydig cells by cAMP-dependent processes [1]. In addition, it is established that calcium is required for these stimulations [2], and that the LH control involves a rise of free cytosolic calcium [3].

Here, we have applied the whole-cell patch-clamp method [4] to investigate the ionic currents in the membrane of single Leydig cells. We demonstrate the presence of two kinds of currents which can be distinguished on the basis of their kinetic properties, their voltage dependence and their sensitivity to intracellular calcium and extracellular blocking agents.

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2. MATERIALS AND METHODS

All experiments were performed on Leydig cells isolated from adult rat testis by an incubation in presence of collagenase [5], followed by a purification through a discontinuous gradient of Percoll® . Cells were plated on plastic petri dishes either previously coated by collagen, for the shorter period of incubation (1–6 h, or directly on the plastic for the longer period (up to 30 h). Cells were cultured in RPMI medium (Flow Lab.) with fetal calf serum (Gibco, 1%). They were either directly used or stimulated with hCG (organon, 15 IU/ml) for 40 min prior to measurement.

Patch-clamp experiments were performed on single cells using the whole-cell recording configuration [4]. The recordings were obtained at room temperature (near 20°C). The capacitance of holder and patch pipettes was compensated with a List EPC 7 amplifier. Once the whole-cell configuration was achieved the cell membrane potential was clamped between −40 and −70 mV. Then

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300–350 ms duration depolarizing or hyperpolarizing pulses were applied at a frequency of 0.1 Hz.

Whole-cell currents were recorded on a magnetic tape recorder and were later replayed for analysis. Current recordings presented herein were not corrected for leakage currents. The capacitive transients were or were not compensated in view of their brevity (see figure legends). No compensation for series resistances (near 10 MΩ; initial pipette resistance between 2 and 5 MΩ) was applied during the experiments. The test potential indicated in the figure legends was corrected for the voltage drop across R_s, calculated at the plateau current, and for the liquid-junction potential between the bath and the pipette solutions. The test potentials were taken into account in *I-V* plots of currents. These curves were constructed from computer programs GRXY and DESS (Poindessault, J.P., unpublished).

The standard extracellular solution contained (mM): 140 NaCl; 5 KCl; 2 $CaCl_2$; 1 $MgCl_2$; 10 Hepes-NaOH: 10 glucose; pH 7.2, 300 mosM. The pipette internal solution was (mM): KCl (added at a concentration such that the total internal concentration was 150); 1 $MgCl_2$; 10 Hepes-KOH; pH 7.2, 280 mosM. This internal solution contained EGTA and $CaCl_2$ to buffer the intracellular free calcium in the concentration range 10^{-9}–10^{-6} M (see figure legends).

In some experiments the blocking effect of

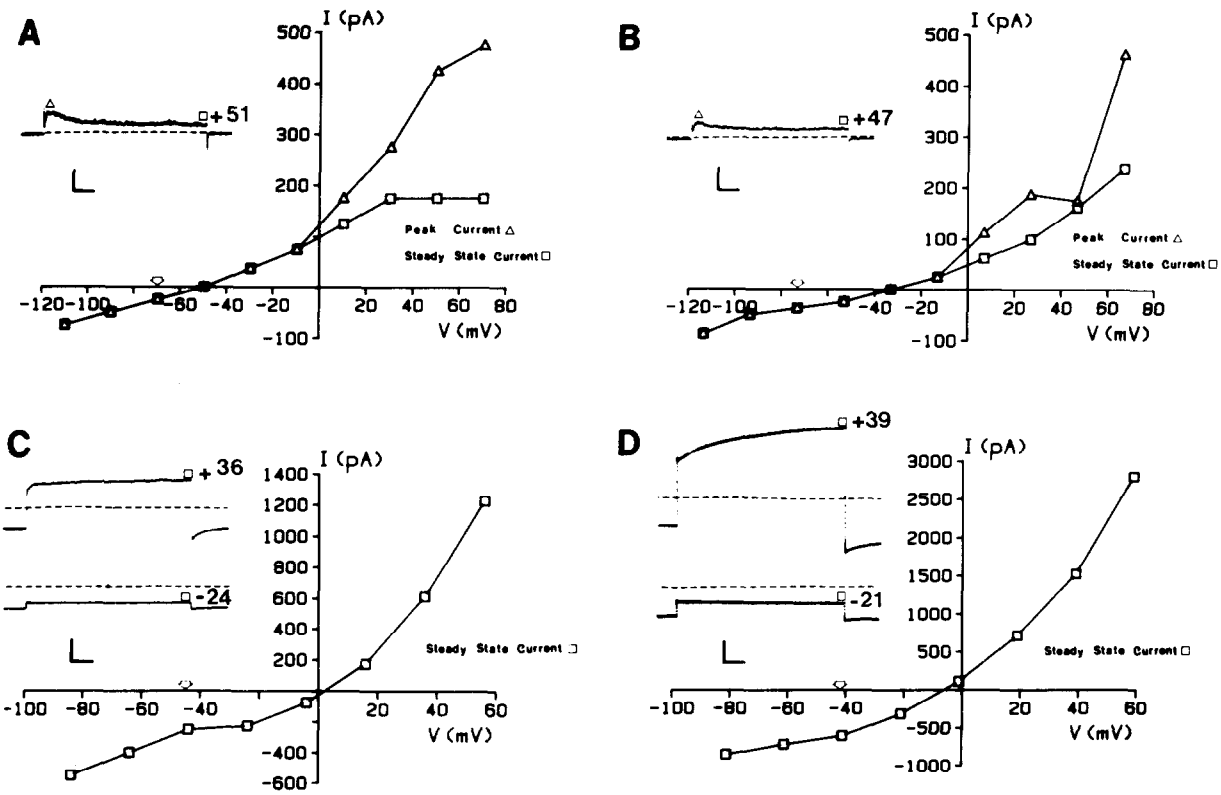

Fig.1. Membrane currents of Leydig cells in standard solution at four internal calcium levels. (A) Approx. 10^{-9} M (0 Ca–0.5 EGTA). (B) 10^{-8} M (1 Ca–11 EGTA). (C) 10^{-7} M (5 Ca–10 EGTA). (D) 10^{-6} M (10 Ca–11 EGTA). Recordings: responses to depolarizing voltage pulses from holding potential to test potentials indicated near the recordings (capacitive transients corrected only in C,D). Dash lines: zero-current levels. Holding currents (pA): A, −25; B, −37; C, −250; D, −600. Calibrations: current, 500 pA; time, 50 ms. *I-V curves:* from the same cells. Arrows: holding potential. Leakage currents were not subtracted since these currents were very small in Leydig cells dialyzed with low calcium media.

tetraethylammonium (TEA, 10 mM) dissolved in the extracellular medium was tested on K^+ current. To study the Cl^- current, an external solution was used to move E_{Cl} from -3 to 40 mV, by replacing 125 mM NaCl by equimolar Na glutamate. TEA and glutamate solutions were applied by a microperfusion system around the test cell.

3. RESULTS

Individual Leydig cells freshly plated on collagen or cultured in RPMI medium over a short time were used for the voltage-clamp experiments. They had a spherical appearance, the characteristic bright ring already described [5] when observed in phase-contrast optics, and an average diameter of 14.5 ± 0.2 μm ($n=41$).

The zero-current potential determined within the second of attaining the whole-cell recording configuration was used as an estimation of the membrane potential. This potential averaged -30.7 ± 2.4 mV ($n=40$). This was higher than the value determined with microelectrode method [5] and was not significantly different in freshly plated and cultured cells, stimulated or not by (15 mIU/ml) hCG.

The cells were then clamped at holding potentials ranging between -40 and -70 mV, and depolarizing voltage steps were applied to bring the membrane potential various test potentials. Fig.1 illustrates typical examples of the recordings obtained from four different cells dialyzed with high K^+ pipette solutions containing various free calcium concentrations.

With the low calcium micropipette solutions (10^{-9}–10^{-8} M), depolarizing voltage steps near -10 mV elicited a small outward current (fig.1A, B). This current reached a peak value in less than 50 ms and then slowly declined towards a steady-state level. The return of potential to the holding potential was not followed by any tail current. The peak amplitude of the outward current and the current at the end of the pulse were plotted as a function of membrane potential. As the membrane potential was made more positive the peak amplitude of the outward current became larger, while negative voltage pulses did not produce any appreciable voltage or time dependent currents.

The maximum peaks of outward current per unit area of the cells were nearly identical for the two calcium buffering solutions [52.8 ± 19.6 μA/cm^2 ($n=5$; 10^{-9} M Ca) compared with 50.9 ± 10.6 μA/cm^2 ($n=7$; 10^{-8} M Ca) for currents elicited at 60 mV test potentials].

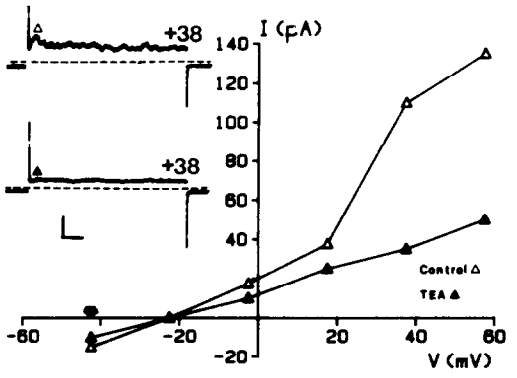

Fig.2. Effects of TEA (10 mM) on outward currents in a Leydig cell dialyzed with a low calcium medium. Recordings: responses to depolarizing voltage steps before (△) and during (▲) addition of TEA. Holding potential: -41 mV. Dashed lines: zero-current levels. Calibrations: currents 100 pA, 50 ms. *I-V* curves: for the peaks of outward current in standard solution (△) and in TEA (▲). TEA caused a 65% reduction of the peak current.

The sensitivity of the outward current to a K^+ channel blocker was tested. Fig.2 shows that TEA (10 mM) caused a reduction of the peak outward current.

Strikingly different currents were measured when the pipette solution contained 10^{-7} and 10^{-6} M calcium. At these calcium concentrations, after the entry into the whole-cell recording, there was a time-dependent shift of the current recorded at the holding potential (holding current). It was inward for negative holding potentials. This current, which was low in 10^{-9} and 10^{-8} M calcium, reached a steady-state level, over 3–5 min, for the higher calcium concentrations (10^{-7}–10^{-6} M).

Fig.1C,D presents the results obtained when positive voltage pulses were applied to cells dialyzed with 10^{-7} and 10^{-6} M calcium. The traces present long-lasting current changes towards a steady-state level, followed by a tail current upon returning to the holding potential. The polarity of the component that developed during the depolarization was dependent upon the test poten-

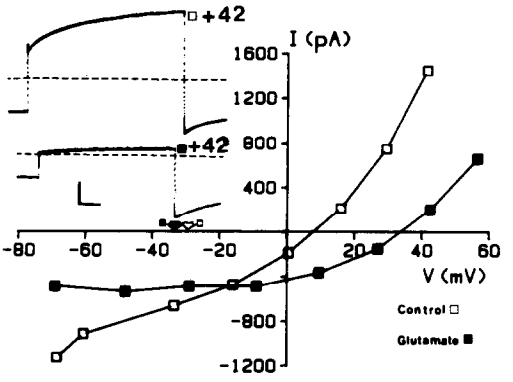

Fig.3. Effect of replacing NaCl (125 mM) in external solution by equimolar Na glutamate on outward currents from Leydig cells dialyzed with 10^{-6} M calcium. Experiments on two different cells. Recordings: responses of cells to depolarizing steps at the same test potentials. Capacitive transients were corrected in both experiments. The outward and holding currents were lower in glutamate (■) while the tail current was somewhat larger. *I-V* curves: from the two cells for the steady-state outward currents in standard (□) and Na glutamate (■) solutions. The reversal potentials were 8 mV in control and 33 mV in Na glutamate solution.

tial. It was inward for negative test potentials, reversed near 0 mV ($E_{Cl} = -3$ mV) and was outward at more depolarized potentials. Its amplitude was quite large in cells dialyzed with 10^{-6} M calcium compared to 10^{-7} M.

As the reversal potential of the current was near E_{Cl}, experiments were performed to shift E_{Cl} from -3 to 40 mV in order to test the selectivity of this calcium-dependent current (fig.3).

When Cl^- was replaced by glutamate the outward relaxation became noisy and was markedly reduced while the inward tail was increased. The corresponding *I-V* curves show that this substitution shifted the reversal potential to more positive values.

Nearly identical results were obtained from unstimulated and hCG-stimulated cells.

4. DISCUSSION

The electrical properties of the Leydig cells of rat were investigated using the whole-cell recording configuration of the patch-clamp method [4]. The present results demonstrate for the first time that two different outward currents can be activated by depolarizing steps applied to the membrane of cells dialyzed by different calcium buffered solutions.

These outward currents can be distinguished from their following properties. The first current which was slowly inactivated during prolonged depolarizations was measured when the internal calcium was near 10^{-9}–10^{-8} M. It was diminished by exposure to TEA. These results suggest that this current was mainly carried by K^+. The second outward current was recorded across the membrane when the cells were dialyzed with high (10^{-7}–10^{-6} M) calcium concentrations. It presented slow kinetics and was always nullified near E_{Cl} indicating high selectivity for Cl^-.

This Ca-dependent Cl^- current was activated by depolarizing pulses when the internal Ca concentration was maintained at 10^{-7} or 10^{-6} M. It has the characteristics of the Ca-dependent Cl^- currents described in lacrimal glands [6,7] or in rod inner segment of the salamander retina [8].

So, in the present study, we show that K^+ is the cation responsible for the outward current evoked by membrane depolarization applied to cell dialyzed with low calcium medium. When the free cytosolic calcium is high, Cl^- becomes the anion responsible for the outward current.

Testosterone secretion from Leydig cells stimulated by secretagogues such as LH is associated with an increase in intracellular calcium as measured by quin 2 fluorescence [3]. Previous intracellular microelectrode studies have provided evidence for the presence of a K^+-conducting pathway in the membrane of the unstimulated cell which is blocked by hCG stimulation [9].

The role of these conducting pathways in the LH/hCg stimulated testosterone secretion of Leydig cells remains speculative and must be clarified.

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REFERENCES

[1] Catt, K.J., Harwood, J.I., Clayton, R.N., Davies, T.F., Chou, V., Katokinemi, M., Noau, K. and Dufau, M.L. (1980) Recent Prog. Hormone Res. 36, 557–622.
[2] Janszen, F.H.A., Cooke, B.A., Van Driel, M.J.A. and Van der Molen, M.J. (1976) Biochem. J. 160, 433–437.
[3] Sullivan, M.H.J. and Cooke, B.A. (1986) Biochem. J. 236, 45–51.
[4] Sakman, B. and Neher, E. (1983) Single Channel Recordings, Plenum, New York.
[5] Joffre, M., Mollard, P., Régondaud, P., Alix, J., Poindessault, J.P., Malassiné, A. and Gargouïl, Y.M. (1984) Pflügers Arch. 401, 239–245.
[6] Marty, A., Tan, Y.P. and Trautmann, A. (1984) J. Physiol. 357, 293–325.
[7] Evans, M.G. and Marty, A. (1986) J. Physiol. 378, 437–460.
[8] Bader, M.G., Bertrand, D. and Schwartz, E.A. (1982) J. Physiol. 331, 253–284.
[9] Joffre, M., Mollard, P., Régondaud, P. and Gargouïl, Y.M. (1984) Pflügers Arch. 401, 246–253.