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Inhibitory effect of internal sodium and Hepes on the voltage-dependent potassium conductance of rat Leydig cells

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Abstract

We used the whole-cell configuration of the patch-clamp technique to investigate modulation of the voltage-dependent K^+ conductance of rat Leydig cells by intracellular pH, Hepes, and Na^+. pH (range 6.8–7.4) has no effect on current. Removal of Na^+ (from 10 to 0 mM) increases the amplitude of K^+ current and abolishes the plateau effect on peak current. Reduction of Hepes (from 25 to 10 mM) increases the amplitude of K^+ current and, simultaneously, shifts the threshold for current activation to the left, but the plateau of current is still present. Removal of Na^+ cancels the potentiating effect of Hepes on frequency of occurrence of time-dependent inactivation of K^+ current. We conclude that the blocking effect of Hepes and Na^+ on K^+ current may presumably involve two distinct mechanisms, but we can not exclude some cooperativity in their ability to inactivate the voltage-gated potassium conductance of rat Leydig cells.

Keywords: Leydig cell; Potassium ion current; Patch clamp; pH; Hepes; Sodium ion

1. Introduction

A voltage-dependent potassium conductance was demonstrated in Leydig cells from mature rat testis [1,2]. This outwardly-rectifying conductance is gradually inhibited by calcium ions when the cytosolic concentration is increased from 1 nM to 100 nM. This conductance is activated at the resting potential. The corresponding currents activate within 50 ms then often show time-inactivation to a steady-state level. This inactivation varies from cell to cell without any dependence on season, age of rat or culture duration [2]. Human chorionic gonadotropin inhibits these currents in a concentration-independent manner, probably via calcium ions [3].

Some potassium channels are modulated by intracellular pH [4–8]. We used the whole-cell patch-clamp technique to study the effect of intracellular pH, Hepes and sodium under conditions where, (1) glutamate substituted for chloride ions in order to minimize the chloride currents, (2) the calcium concentration was maintained at pCa > 9 with EGTA in order to study the calcium-inhibited outwardly-rectifying potassium conductance.

2. Materials and methods

2.1. Preparation of Leydig cells

Leydig cells were freshly isolated from testes of mature rats WISTAR (AF) as previously described

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[2]. Testes were mechanically and enzymatically dissociated in a solution containing collagenase (Serva, Germany) and trypsin inhibitor (Sigma, USA). Leydig cells were purified by density centrifugation on a discontinuous Percoll gradient. They were plated on 35-mm plastic petri dishes and cultured in modified RPMI 1640 medium (Gibco, UK). Cells were used 4 to 36 h after purification.

2.2. Recording conditions

The tight-seal whole-cell configuration of the patch-clamp technique [9] was performed with a patch-clamp amplifier (RK300, Bio-logic, France). Pipettes of 2–5 MΩ were formed from borosilicate glass capillary tubing (GC150-TF10, Clark Electromedical Inst., UK), coated with sylgard 184 (Dow Corning, Belgium) then fire-polished. Seal resistances ranged from 4 to 15 GΩ. Pipette capacitance was compensated electronically in cell-attached mode. Series resistance and membrane capacitance that are measured in the whole-cell mode, averaged 11.1 $\pm$ 0.3 MΩ and 23.9 $\pm$ 1.0 pF, respectively ($\pm$S.E.M. for 73 cells). Protocols and data acquisition were performed with an IBM microcomputer (PC XT286) through an A/D-D/A conversion board (TM-40, Teckmar, USA) and a specific software (PCLAMP 5.5.1., Axon Inst., USA). Cell currents were low-pass filtered at 3.3 kHz and digitized on-line at 4 kHz. In current–voltage curves, the current values are corrected for linear leakage and divided by membrane capacitance before being averaged as mean $\pm$S.E.M. for n cells. All membrane voltages are corrected for the liquid junction potential which is equal to +6 mV. Statistical calculations were performed with unpaired Student's t-tests (Fig. P 6.0, Biosoft USA). All experiments were performed at room temperature (20–23°C).

2.3. Solutions

Petri dishes were rinsed several times with the bathing solution just before experiment (see Table 1). Pipettes were filled with internal solutions as indicated for each experiment (see Table 1). External pH was adjusted to 7.40 $\pm$ 0.05 with NaOH, and internal pH to 7.20 $\pm$ 0.05 with KOH, except when mentioned. Osmolarities were controlled to 300 $\pm$ 5 mosmol/kg H_2O (external solution) and 280 $\pm$ 5 mosmol/kg H_2O (internal solution).

2.4. Chemicals

Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), EGTA (ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid) and ATP-Mg (adenosine 5'-triphosphate, magnesium salt) were purchased from Sigma (St. Louis, MO, USA); NaGlu (L-glutamic acid, monosodium salt) and KGlu (L-glutamic acid, monopotassium salt monohydrate) from Fluka (Buchs, Switzerland); magnesium sulfate heptahydrate from Merck (Darmstadt, Germany); calcium sulfate dihydrate from Prolabo (Paris, France).

3. Results

In order to strongly buffer the intracellular pH we used internal solutions containing 25 mM Hepes. Variations of pH from 6.8 to 7.7 do not modify the amplitudes and the activation threshold of the outwardly-rectifying potassium current (Fig. 1). Outward rectification and the plateau of currents observed at high potentials are present whatever the pH.

By contrast, Hepes has an inhibitory effect on potassium currents (Fig. 2). For the same pH of 7.2 and the same sodium concentration of 10 mM, the

Table 1
Ionic concentrations (in mM) of external and internal solutions

Soln.	K^+	Na^+	Mg^{2+}	Ca^{2+}	Glu^-	SO_4^{2-}	EGTA	ATP	Hepes
A	5	156	2	2	157	2	–	–	10
1	134	10	2	–	120	4	10	3	10
2	133	10	2	–	117	4	8	3	25
3	133	–	2	–	117	4	8	3	25

A: external solution; 1–3: internal solutions.

Table 2
Summary of the variations in time-dependent inactivation of currents measured at +74 mV

Soln.	Hepes (mM)	Na^+ (mM)	Frequency of occurrence	Mean ± S.E.M.	Mini	Maxi
1	10	10	8/15	31.2 ± 3.4%	0%	47.9%
2	25	10	29/29	50.9 ± 2.5%	26.5%	77.1%
3	25	0	1/5	–	0%	21.7%

Frequency of occurrence is expressed as the number of cells presenting inactivating currents at a potential of +74 mV dividing by the total number of cells tested. The mean of percentage of inactivation is calculated from the cells showing inactivating currents. Mini and Maxi correspond to the extreme values of percentage of inactivation.

amplitudes of peak-current are significantly reduced when the Hepes concentration is increased from 10 to 25 mM ($P < 0.01$ at potentials from −46 to +114 mV, Fig. 2C), and the threshold for current activation is shifted to the left. Moreover, all the 29 cells dialysed with 25 mM Hepes present a time-dependent inactivation of currents at +74 mV against 8 out of 15 cells dialysed with 10 mM Hepes; an inactivation that ranges from 26.5 to 77.1% of peak current in the presence of 25 mM Hepes, and from 0 to 47.9% in the presence of 10 mM Hepes (Fig. 2A, B; Table 2).

When sodium ions are removed from the internal solution containing 25 mM Hepes, amplitudes of peak-current are significantly larger than those recorded in the presence of 10 mM Na^+ ($P < 0.05$ at +34 mV, $P < 0.01$ from +54 to +114 mV; Fig. 3). The threshold for current activation is not modified, but outward rectification is increased and the plateau of current is abolished. Only 1 out of 5 cells dialysed with Na-free solution presents a time-dependent inactivation of currents (Table 2).

4. Discussion

Using the whole-cell voltage-clamp technique, we show that the voltage-dependent potassium conductance of rat Leydig cells is insensitive to intracellular pH. But it appears that Hepes, used as pH buffer, inhibits the potassium currents by about 50% when the concentration is increased from 10 to 25 mM.

Hepes, which presents a good profile for a biological buffer [10] is known to have some side effects, notably on membrane ionic currents. For example, it inhibits acetyl choline-induced currents in *Helix* neurons [11]; it induces flickering in a chloride channel from *Drosophila* neurons [12] and reduces the conductance of the outwardly-rectifying anion channel which may be involved in epithelial chloride transport and cell volume regulation [13]. This report provides the first demonstration of an effect of Hepes on potassium currents in Leydig cells from mature rat testis. In T lymphocytes, where the sensitivity to pH

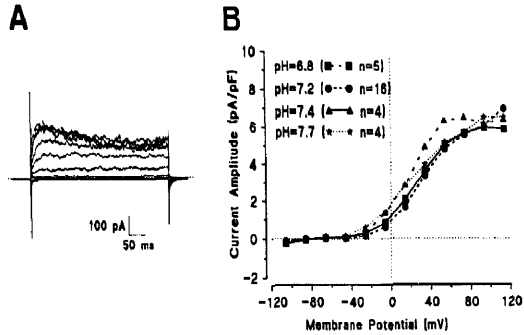

Fig. 1. Effect of intracellular pH on potassium currents. (A) Current traces elicited by voltage pulses of 500 ms duration, applied every 20 s, from −46 mV to potentials ranged from −106 to +114 mV, in 20 mV increments. The pH of internal solution was buffered at 7.2 (Soln. A/2) but no difference was observed whatever the pH. (B) Current–voltage curves for the currents elicited by the same protocol as in (A) in cells dialysed with solution containing 25 mM Hepes (Soln. A/2). pH was adjust to 6.8, 7.2, 7.4, or 7.7, before each experiment.

of the delayed-rectifier potassium channel is studied. Hepes, in absence of internal sodium, has no effect at concentrations ranging from 10 to 50 mM [7].

We also show that intracellular sodium inhibits the voltage-gated potassium currents in rat Leydig cells. This inhibition is significant at potential above +14 mV so that an inward rectification occurs for the highest potentials. Such effect of sodium has already been observed on the calcium-activated potassium channels from bovine adrenal chromaffin cells [14–16] and on the delayed-rectifying potassium currents in Schwann cells from rabbit sciatic nerve [17]. In these tissues, sodium ions induce flickering in the potassium channels and, as in rat Leydig cells, inward rectification of currents at high potentials.

Because removal of Na^+ increases the amplitude of K^+ current at positive potentials and abolishes the plateau effect on peak current, while reduction of Hepes increases the amplitude of K^+ current and, simultaneously shifts the threshold for current activation to the left, without any modification of the plateau of current which is still present, we conclude that the blocking effect of Hepes and Na^+ on K^+ current may presumably involve two distinct mechanisms. But, because removal of Na^+ cancels the potentiating effect of Hepes on frequency of occurrence of time-dependent inactivation of K^+ current, we can not exclude some cooperativity in their ability to inactivate the voltage-gated potassium conductance of rat Leydig cells. This study make conspicuous the

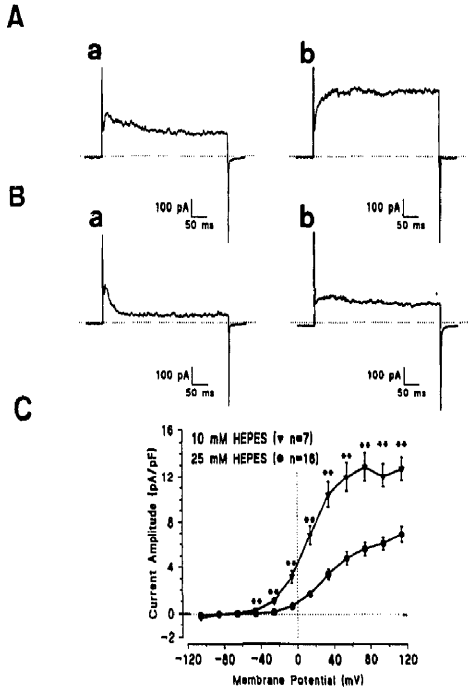

Fig. 2. Effect of Hepes on potassium currents, in presence of 10 mM sodium. (A) Current traces elicited by voltage pulses of 500 ms duration, applied from −46 mV to +74 mV, in two cells dialysed with 10 mM Hepes (Soln. A/1) and presenting the two extreme behaviours in time-inactivation (a and b). (B) Current traces elicited as in (A) in two cells dialysed with 25 mM Hepes (Soln. A/2) and presenting the two extreme behaviours in time-inactivation (a and b). (C) Current–voltage curves for the currents elicited by the same protocol as in Fig. 1A in cells dialysed with 10 mM Hepes (Soln. A/1; $n = 7$) or 25 mM Hepes (Soln. A/2; $n = 16$).

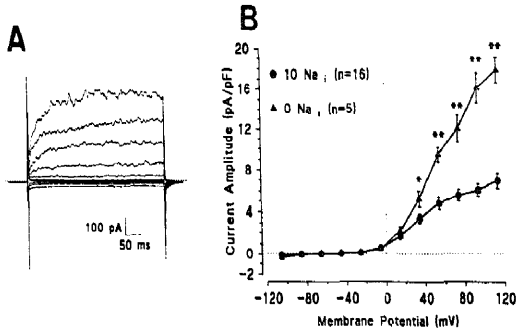

Fig. 3. Effect of sodium ions on potassium currents, in presence of 25 mM Hepes. (A) Current traces elicited by voltage pulses of 500 ms duration, applied every 20 s, from −46 mV to potentials ranged from −106 to +114 mV, in 20 mV increments. The cell was dialysed with Na^+-free solution (Soln. A/3). For comparison with currents elicited in a cell dialysed with Na^+-riched solution (Soln. A/2), see Fig. 1A. (B) Current–voltage curves for the currents elicited by the same protocol as in (A) in cells dialysed with Na^+-riched solution (Soln. A/2; $n = 16$) or Na^+-free solution (Soln. A/3; $n = 5$).

unexpected effect of Hepes, a frequently used pH buffer.

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