A slowly inactivating calcium current works as a calcium sensor in calcitonin-secreting cells

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Calcitonin (CT)-secreting cells (C-cells) are remarkably sensitive to changes in the extracellular Ca^{2+} concentration. In order to detect the mechanism by which C-cells monitor Ca^{2+}, we compared a C-cell line responding to Ca^{2+} (rMTC cells) with another one known to have a defect in this Ca^{2+} signal transduction (TT cells). Rises of the Ca^{2+} concentration caused rMTC cells to depolarize and/or elicited spontaneous action potentials. Under voltage-clamp conditions, rMTC cells showed a slowly decaying Ca^{2+} inward current which was sensitive to dihydropyridines but not to Ni^{2+} at a low concentration. In contrast, the 'defective' TT cells neither depolarized nor fired action potentials with high Ca^{2+}; they only exhibited an Ni^{2+}-sensitive, transient Ca^{2+} current. The data strongly suggest that the slowly inactivating Ca^{2+} current is a prerequisite for Ca^{2+}-sensitivity of C-cells and that fast inactivating channels are not sufficient to act as sensors of the extracellular Ca^{2+} concentration.

Calcitonin-secreting cell; rMTC cell; TT cell; Calcium current

1. INTRODUCTION

Calcitonin (CT)-secreting cells (C-cells) are preferentially located in the thyroid gland and are considered to play an important role in the complex regulatory network of Ca^{2+} homeostasis. Previous reports on the permanent C-cell line rMTC [1,2] revealed a close correlation between the extracellular Ca^{2+} concentration and both the intracellular Ca^{2+} concentration [3] and CT release. The Ca^{2+}-dependent CT release was blocked by nitrendipine [4] or verapamil [5], mimicked by Bay K 8644 [4,6] and potentiated by (+)202–791 [7]. In contrast, C-cells of the TT-line [8], due to an unknown defect, are unable to respond to Ca^{2+}, but CT secretion rises after electropermeabilization [9]. Although these findings have suggested a prominent role of voltage-dependent Ca^{2+} channels [4,7,10] for the Ca^{2+} sensitivity of C-cells, no electrophysiological data supporting this hypothesis are yet available. Here we report on whole cell recordings of voltage-dependent Ca^{2+} currents in rMTC and TT cells.

2. MATERIALS AND METHODS

2.1. Cell culture

Rat MTC cells (rMTC 44-2 C-cell line) were grown in monolayer culture using DMEM (Biochrom, Berlin, FRG) supplemented with 15% horse serum and 2.5% fetal calf serum (Gibco, Paisley, UK). Human MTC cells (TT cells, hMTC C-cell line) were grown in the same way except for the use of RPMI-1640 medium (Gibco, Paisley, UK) supplemented with 16% fetal calf serum.

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2.2. Secretion experiments

Confluent rat and human C-cells were preincubated with serum-free DMEM or RPMI medium for 2 h, washed twice with PBS buffer and incubated again with medium containing test agents or vehicle for 1 h. Then medium was removed and stored at $-20°C$ until assayed for immunoreactive CT [12]. CT secretion was standardized to cellular protein content.

2.3. Electrophysiology

For electrophysiological investigations, cells were cultured on small glass slides (density 2–5 cells/mm^2). After transfer into a chamber (0.2 ml), the attached cells were superfused at a constant rate of about 5 ml/min. The whole cell membrane currents were measured according to the method described by Hamill et al. [11] for special modifications (see [13]). The patch electrodes had an average resistance of 5 MΩ (open diameter about 1 μm), which allowed to obtain GΩ seals within about 30 s. After disruption of the membrane patch under the tip of the patch pipette, a whole cell configuration was obtained, suitable for measuring membrane currents under voltage-clamp conditions (see [11]). Or, alternatively and in order to avoid a major disturbance of the cytoplasma, e.g. change of the intracellular Ca^{2+} buffering capacity, we assessed the cytoplasma using the nystatin method [14,15].

2.4. Solutions

External solution E1 contained (in mM): 135 NaCl, 1.2 CaCl$_2$, 1 MgCl$_2$, 5.4 KCl, 10 glucose and 10 Hepes (pH 7.4 with NaOH, 37°C). Solutions E2–E4 contained: 135 or 125 TEA-Cl, 1 MgCl$_2$, 10 glucose, 10 Hepes (pH 7.4 with TEA-OH, 37°C), TTX (200 nM) and 1.2 CaCl$_2$ (solution E2) or 10.8 CaCl$_2$ (solution E3) or 10.8 BaCl$_2$ (solution E4). Solutions E5–E6 contained: 135 or 125 NaCl, 1 MgCl$_2$, 5.4 CsCl, 10 glucose, 10 Hepes (pH 7.4 with NaOH, 37°C), 200 nM TTX and 1.2 BaCl$_2$ (solution E5) or 10.8 BaCl$_2$ (solution E6). Pipette solution I1 contained (in mM): 90 K-aspartate, 50 KCl, 4 MgCl$_2$, 10 Hepes (pH 7.4 with KOH, 37°C), 3 Na$_2$-ATP and was supplemented with freshly prepared nystatin (100 200 μg/ml). I2 contained: 120 CsOH, 120 L-aspartate, 20 CsCl, 4 MgCl$_2$, 3 Na$_2$-ATP, 10 Hepes (pH 7.4 with TEA-OH) and was supplemented with freshly prepared nystatin (100 200 μg/ml). I3 contained: 100 CsCl, 40 CsOH, 4 MgCl$_2$, 3 Na$_2$-ATP, 10 Hepes (pH 7.4 with CsOH, 37°C), 10 EGTA, 6.0 CaCl$_2$; the calculated free concentration of Ca^{2+} in this solution was 0.1 μM.

2.5. Statistics

Data are presented as the mean ± SE. Statistical significance was assessed by the Wilcoxon rank sum test.

3. RESULTS

3.1. Secretion experiments

Table I shows the effects of high Ca^{2+} and the Ca^{2+} channel agonist Bay K 8644 on CT release from rMTC and TT cells. Rising Ca^{2+} from 1.1 to 2.0 mM or adding 10 μM Bay K 8644 increased CT secretion from rMTC cells but did not affect CT release from TT cells, agreeing with previous reports [6,9].

3.2. Spontaneous action potentials

rMTC cells exhibited resting potentials of -44.5 ± 1.6 mV ($n = 38$). Rising the Ca^{2+} concentration from 1.2 to 1.8 mM elicited spontaneous action potentials (Fig. 1 1A) and/or depolarized the cells by 12.1 ± 2.3 mV ($n = 9$). The action potentials evoked by high Ca^{2+} could be reversibly suppressed by the Ca^{2+} channel blocker isradipine (PN 200-110, 1 mM) (Fig. 1C). At 1.2 mM Ca^{2+}, 1 mM Bay K 8644 often induced a few initial spikes but then led to a continuous depolarisation by 16.3 ± 2.9 mV ($n = 10$). Washing out Bay K 8644 repolarized the cells (Fig. 1E). In contrast, the resting membrane potential of TT cells was affected neither by high Ca^{2+} (Fig. 1B) nor isradipine (Fig. 1D) nor Bay K 8644 (Fig. 1F); their resting potential stayed at -37.9 ± 1.8 mV ($n = 13$).

3.3. Ca^{2+} currents during voltage-clamp steps

To measure the Ca^{2+} currents, K^+ and Na^+ currents were blocked with Cs^+, TEA and tetrodotoxin. Under these conditions and with 1.2 mM Ca^{2+} as divalent charge carrier, rMTC cells exhibited slowly inactivating inward currents (Fig. 2A) which had a maximal current density of 9.3 ± 0.7 pA/pF ($n = 12$) at -10 mV. The current-voltage (IV) relationship revealed a threshold at about -50 mV and an apparent reversal potential at 30 mV. Increasing the Ca^{2+} concentration to 10.8 mM raised the maximal current 2.5-fold and shifted the threshold of the IV-curve to about -40 mV (data not shown). Semilogarithmic plotting of the Ca^{2+} current-inactivation during depolarisation pulses for 3 s [16] revealed two inactivation constants τ_1 and τ_2 in rMTC
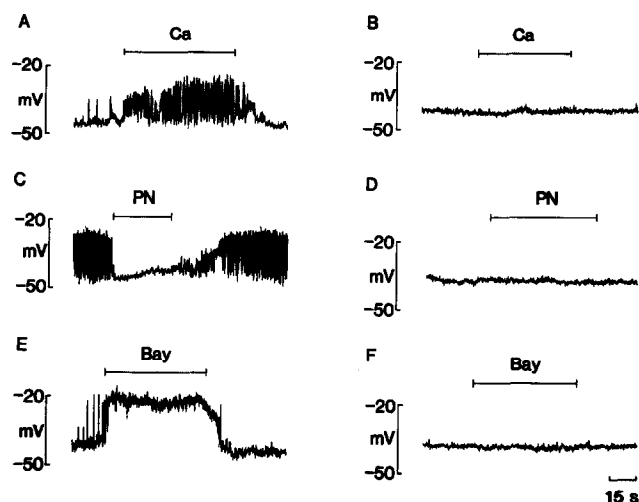

Fig. 1. Effects of Ca^{4+}, a Ca^{4+} channel blocker and an agonist on membrane potentials in C-cells. The effects of 1.8 μM Ca^{2+} (Ca) (A and B), 1 μM isradipine (PN) (C and D) and 1 μM Bay K 8644 (Bay) (E and F) on membrane potentials of rMTC (left) and TT (right) cells are shown. The substances were added as indicated by the horizontal lines. Solutions: pipette solution I1 and external solution E1. External solution E1 with 1.8 instead of 1.2 μM Ca^{2+} was applied as marked in (A) and (B) and throughout in (C) and (D).

cells. With 1.2 mM Ca^{2+} and at 37°C, τ_1 amounted to 168 ± 11 ms and τ_2 to 4.9 ± 0.7 s ($n = 5$) for depolarisation steps from -80 to -10 mV.

Under 1.2 mM Ca^{2+}, TT cells showed negligibly small currents. At 10.8 mM Ca^{2+}, transient inward currents were detectable which completely inactivated within about 40 ms (Fig. 2B). Compared to the current of rMTC cells, the current density was about 4.5-fold smaller and the IV curve was obviously shifted to the left. In agreement with the described properties of the

Table I

Effects of Ca^{2+} and Bay K 8644 on calcitonin secretion.

	Calcitonin release	
	rMTC cells (pg·mg protein^{-1}·h^{-1})	TT cells (ng·mg protein^{-1}·h^{-1})
Control	126 ± 5.8	4.58 ± 0.32
10 μM Bay K 8644	229 ± 8.0*	4.70 ± 0.27[ns]
2 mM Ca^{2+}	257 ± 10.2*	4.37 ± 0.24[ns]

Basal Ca^{2+} concentration was 1.1 mM. Mean ± SEM ($n = 5$).
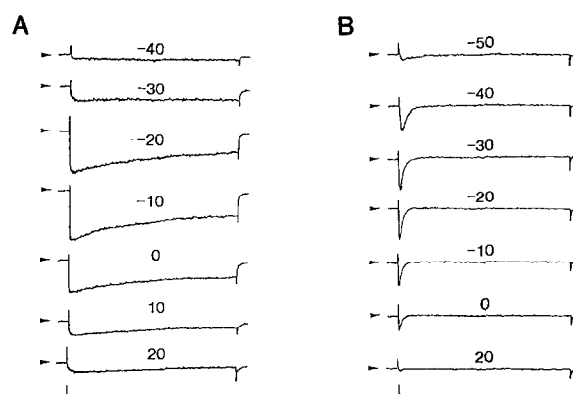*$P < 0.01$; [ns]not significant to control

Fig. 2. Whole-cell recordings of Ca^{2+} currents in C-cells. Original current traces of rMTC (A) and TT (B) cells are shown during 300 ms long voltage clamp pulses from -80 mV to various test potentials as indicated by the numbers. The membrane patch under the tip of the pipette was disrupted and free access to the cytoplasm was obtained. Solutions: pipette solution I3 for both rMTC and TT cells; external solutions E2 (1.2 μM Ca^{2+}) for rMTC and E3 (10.8 μM Ca^{2+}) for TT cells. Vertical and horizontal calibration marks correspond to 30 pA and 30 ms. Cell capacity: 12.1 pF (A) and 15.9 pF (B).
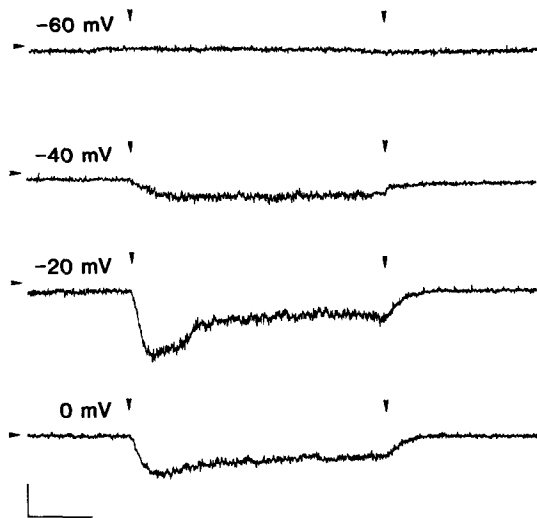
Fig. 3. Effect of Ba^{2+} on inward currents in a single rMTC cell at various holding potentials. The rMTC cell was voltage-clamped at the indicated potentials for 2 min. For the period marked in between the vertical arrows Ba^{2+} was raised from 1.2 mM (solution E5) to 10.8 mM (solution E6); pipette solution I2. Horizontal arrows mark the zero current level. Calibration marks correspond to 15 s and 20 pA. Cell capacity: 14.3 pF.

Fig. 4. Effects of Ca^{2+} channel blockers and an agonist on Ca^{2+} currents in C-cells. The effects of 10 μM Ni^{2+} (Ni) (A and B), 1 μM isradipine (PN) (C and D) and 1 μM Bay K 8644 (Bay) (E and F) on currents of rMTC (left) and TT (right) cells are shown. CON refers to the controls. Test pulses from -60 to 0 mV and from -80 to -30 mV were applied to rMTC and to TT cells, respectively. Solutions: Pipette solution I3 and external solution E4. Arrows mark the zero current level. In (A, C and E), calibration marks correspond to 20 ms and 100 pA, in (B, D and F) to 10 ms and 30 pA.

transient T-type Ca^{2+} currents [17,18], the threshold was about -60 mV and the maximum (5.1 $\pm$ 0.5 pA/pF, $n = 9$) occurred at about -30 mV.

The permeability ratio of Ba^{2+}/Ca^{2+} (10.8 mM) amounted to 2.1 $\pm$ 0.2:1 ($n = 5$) for Ca^{2+} channels in rMTC cells and to 1.1 $\pm$ 0.1:1 ($n = 4$) for Ca^{2+} channels in TT cells. The midpoint voltage of the steady-state inactivation curve of the Ca^{2+} current as measured with 10.8 mM Ca^{2+} was -24 ± 2.5 mV ($n = 5$) for rMTC cells and -59 ± 2.9 mV ($n = 4$) for TT cells.

3.4. Effect of Ba^{2+} on inward currents

To provide direct evidence for a steady-state conductivity of Ca^{2+} channels underlying the Ca^{2+} sensitivity of rMTC cells, we voltage-clamped C-cells at fixed holding potentials for several minutes and measured the current response to rises of Ba^{2+} from 1.2 to 10.8 mM. At -40 mV, rMTC cells produced an inward current which was maximal (23.0 $\pm$ 8.6 pA, $n = 9$) after about 5 s and then slowly decayed. Fig. 3 3 demonstrates the voltage dependence of this inward current for potentials between -60 to 0 mV. The elicited Ba^{2+} inward current displayed a U-shaped voltage dependence with a threshold of about -50 mV. In contrast to rMTC cells, TT cells failed to display any inward current in analoguous experiments (data not shown).

3.5. Pharmacological characterization

The difference between rMTC and TT cells with respect to their Ca^{2+} currents became more apparent by their different pharmacology. Ni^{2+} at low concentrations is known to specifically block T-type currents
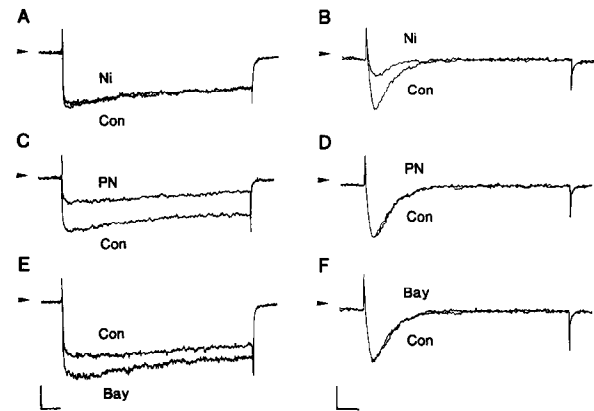
[17]. In line with these reports, the fast inactivating Ca^{2+} current of TT cells was inhibited by 65 $\pm$ 3% ($n = 5$) under 10 μM Ni^{2+} (Fig. 4B); but the slowly inactivating current of rMTC cells was not or only minimally affected (Fig. 4A). A reverse sensitivity was found in the case of dihydropyridines (Fig. 4C-F). Isradipine (PN 200-110, 1 μM) suppressed the Ca^{2+} current of rMTC cells by 57 $\pm$ 5% ($n = 6$) and the Ca^{2+} channel agonist Bay K 8644 (1 μM) stimulated it by 34 $\pm$ 4% ($n = 4$). Both isradipine and Bay K 8644 did not affect the current of TT cells ($n = 7$).

4. DISCUSSION

A major role of dihydropyridine-sensitive Ca^{2+} currents for the Ca^{2+} sensitivity of C-cells has been suggested by the effects of Ca^{2+}, organic Ca^{2+} channel blockers and agonists on calcitonin secretion and cytosolic Ca^{2+} concentration in C-cells. (i) The Ca^{2+}-induced calcitonin release is blocked by Ca^{2+} channel blockers [4,5,10]. (ii) The calcitonin release is stimulated by Ca^{2+} channel openers [4,6,10,12]. (iii) The intracellular Ca^{2+} is highly dependent on the extracellular Ca^{2+} concentration [3]. (iv) The intracellular Ca^{2+} rises with Bay K 8644 and falls with nifedipine [6]. (v) Increasing the extracellular K^+ concentration causes a depolarisation, an increase in the cytosolic Ca^{2+} and a subsequent calcitonin release [1,3,19].

Our electrophysiological studies provide direct evidence for a voltage-dependent, dihydropyridine-sensitive, long lasting Ca^{2+} current in rMTC-cells and its essential role in the Ca^{2+} sensitivity of C-cells as

evidenced by C-cells of the 'defective' TT cell line which lack this Ca^{2+} current and are unable to regulate calcitonin secretion in response to Ca^{2+}; the fast inactivating Ca^{2+} current that can be detected in TT cells fails to substitute as sensor of the extracellular Ca^{2+} concentration. The differing Ca^{2+} sensitivity of rMTC and TT cells cannot be attributed to species differences (rat and human, respectively), as normal human C-cells and several primary cultures of human C-cell carcinoma have been shown to respond to Ca^{2+} [10,20,21].

To monitor the extracellular Ca^{2+} concentration, C-cells need to have a steady state conductivity for Ca^{2+}. Voltage-clamping rMTC cells near their resting potential demonstrated a steady state-inward current through Ca^{2+} channels which depended on the concentration of the divalent charge carrier. Analyzing the Ca^{2+} current-inactivation we determined a fast (168 ms) and a slow inactivation time constant of 4.9 s. With physiological Ca^{2+} concentrations and at 37°C, inactivation constants as long as 4.9 s have not been reported for other cells [22], e.g. in cardiocytes, inactivation occurs within about 100 ms [23]. Whether the two inactivation time constants are due to different inactivated states of the Ca^{2+} channel [24] or the presence of different types of Ca^{2+} channels remains to be evaluated.

C-cells are known to generate tetrodotoxin- and D600-sensitive action potentials [19,25]. Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels apparently plays an important role therein. Increasing Ca^{2+} or adding Bay K 8644 induced action potentials and/or depolarized rMTC cells; isradipine suppressed the Ca^{2+}-evoked action potentials. In addition, the similarity between the activation threshold of the Ca^{2+} current and the resting potential of rMTC cells argues for a role of the slowly inactivating Ca^{2+} current in the generation of spontaneous activity (compare with [26]). T-type Ca^{2+} currents regarded to be involved in spontaneous activity in other cell types [27,28] were not seen in rMTC cells. Moreover, TT cells which exhibited only fast inactivating Ca^{2+} currents did not display spontaneous action potentials. Thus T-type Ca^{2+} currents by themselves seem not to be sufficient to generate pacemaking activity.

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