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Extracellular Ca^{2+} Sensing in C-Cells and Parathyroid Cells

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An essential function of C-cells and parathyroid cells is to monitor the extracellular Ca^{2+} concentration. The Ca^{2+}-dependent secretion of calcitonin (CT) and parathyroid hormone is known to be mediated by corresponding changes in the intracellular Ca^{2+} concentration. To address the question of whether Ca^{2+} influx through voltage-dependent Ca^{2+} channels couples the extracellular to the intracellular Ca^{2+}, we applied the patch clamp technique to C-cells of the rMTC 44-2 cell line and to parathyroid cells of the PT-r cell line. The rMTC cells displayed dihydropyridine-sensitive, voltage-dependent, high-threshold Ca^{2+} channels which allowed ion influx even at the resting potential of about –40 mV. Increases of the concentration of the extracellular divalent cation or adding the Ca^{2+} channel agonist Bay K 8644 stimulated the steady state ion influx. In contrast, PT-r cells exhibited only fast inactivating, low-threshold Ca^{2+} channel currents with no steady state conductivity for Ca^{2+} at the resting potential of around –40 mV. We conclude that dihydropyridine-sensitive Ca^{2+} channels allow steady state transmembranous Ca^{2+} influx in C-cells, thereby increasing the cytosolic Ca^{2+} and CT secretion. Parathyroid cells, however, lack long-lasting Ca^{2+} channel currents and obviously sense the extracellular Ca^{2+} concentration by other mechanisms. (Henry Ford Hosp Med J 1992;40:303-6)

Calcitonin (CT) secretion from parafollicular cells (C-cells) of the thyroid gland and parathyroid hormone (PTH) release from cells of the parathyroid gland are remarkably sensitive to changes of the extracellular Ca^{2+} concentration (1,2). Since the cytosolic Ca^{2+} concentration is the major intracellular messenger for Ca^{2+}-dependent hormone secretion from both cell types, the coupling of extra- to intracellular Ca^{2+} appears to be an essential mechanism for the Ca^{2+} sensitivity. In C-cells, this is accomplished by dihydropyridine-sensitive Ca^{2+} channels as suggested from secretion studies and measurements of the cytosolic Ca^{2+} (3-6). In parathyroid cells, however, the role of dihydropyridine-sensitive Ca^{2+} channels for the Ca^{2+} sensitivity is less clear (7,8). In the present study we performed patch clamp experiments on C-cells of the Ca^{2+}-sensitive cell line rMTC (9) and on parathyroid cells of the Ca^{2+}-sensitive cell line PT-r (10). We found that the Ca^{2+}-sensitivity of C-cells relied on the steady state Ca^{2+} conductivity of dihydropyridine-sensitive Ca^{2+} channels. However, parathyroid cells exhibited only fast inactivating dihydropyridine-resistant Ca^{2+} channels and no steady state Ca^{2+} conductivity.

Materials and Methods

Cell culture

C-cells of the rat medullary thyroid carcinoma (MTC) cell line rMTC 44-2 (9,11) were grown in monolayer culture using Dulbecco's modified Eagle's minimal essential medium (DMEM) (Biochrom, Berlin) supplemented with 15% horse serum and 2.5% fetal calf serum (Gibco, Paisley, UK). Parathyroid cells of the rat parathyroid cell line PT-r (10) were cultured in a calcium- and magnesium-free mixture (1:1) of Coon's modified Ham's F-12 and DMEM supplemented with 5% calf serum (Gibco, Paisley, UK), 1% Nutridoma-SP (Boehringer Mannheim), 100 units of penicillin per mL, 0.1 mg of streptomycin per mL, 1.0 mM $CaCl_2$, and 0.5 mM $MgCl_2$.

Electrophysiology

Cells grown on small glass slides (density about 1,000 cells/mm^2) were transferred into a perfusion chamber (0.2 mL) mounted on an inverted microscope. The cells were superfused with bath solution 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 (12) or, to avoid major disturbances of the cytoplasm, by the perforated patch technique (13). The patch electrodes had a resistance of 3 to 6 MOhm (open diameter about 10^{-6} M), which allowed the obtaining of GOhm seals within 5 to 50 sec.

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). External solution E2 contained: 125 choline-C1, 5mM $BaCl_2$, 1 $MgCl_2$, 5.4 CsCl, 10 glucose, 10 Hepes (pH 7.4 with TEA-OH, 37 °C). Pipette solution I1 contained (in mM): 90 K-aspartate, 50 KCl, 4 $MgCl_2$, 3 Na_2-ATP, 10 Hepes (pH 7.4

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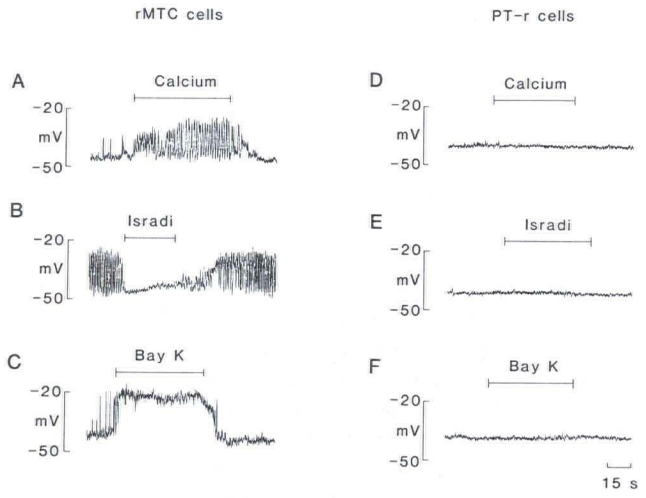

Fig 1—Effects of extracellular Ca^{2+}, isradipine, and Bay K 8644 on membrane potentials in C-cells and parathyroid cells. Where marked by bars, 1.8 mM Ca^{2+} (calcium) or 10^{-6} M isradipine (Isradi) or 10^{-6} M Bay K 8644 (Bay K) were added to rMTC cells (left) or PT-r cells (right). Solutions: internal solution I1 and external solution E1. E1 with 1.8 instead of 1.2 mM Ca^{2+} was applied as indicated in (A) and (D) and throughout in (B) and (E).

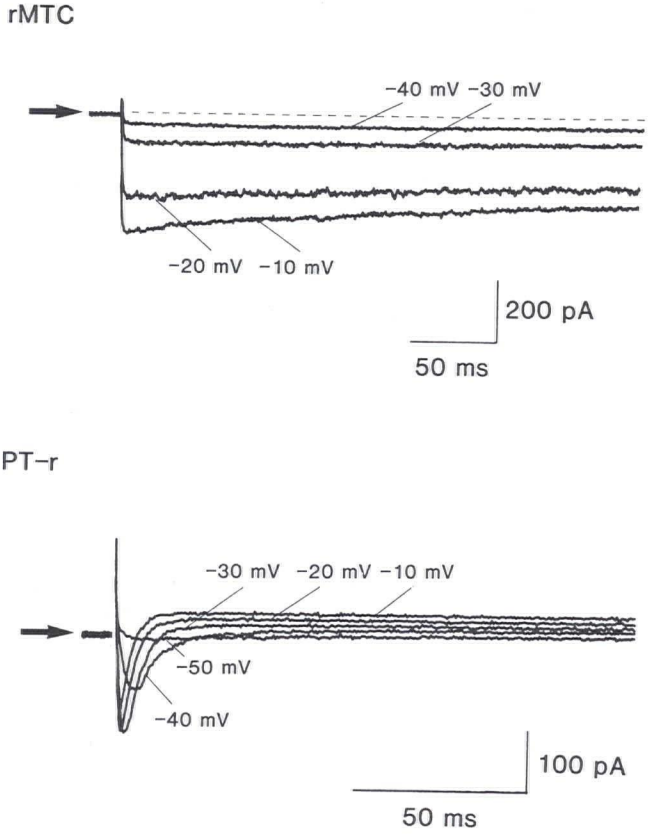

Fig 2—Whole-cell recordings of Ca^{2+} channel currents in C-cells and parathyroid cells. Ca^{2+} channel currents as elicited by test pulses to the various indicated potentials from a holding potential of –80 mV are shown for rMTC (top) and PT-r cells (bottom). Solutions: pipette solution I2; external solution E2. The horizontal arrows mark the respective zero current level.

with KOH, 37 °C), and was supplemented with freshly prepared nystatin (0.1 to 0.3 mg/mL). Internal solution I2 contained: 140 CsCl, 4 $MgCl_2$, 3 Na_2-ATP, 10 Hepes (pH 7.4 with Cs-OH, 37 °C), and, where indicated, was supplemented with freshly prepared nystatin (0.1 to 0.3 mg/mL).

Materials

Bay K 8644 was purchased from Calbiochem, Frankfurt, Germany, and (+) PN 200-110 (isradipine) was a gift of Sandoz (Basel, Switzerland).

Statistics

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

Results

Plasma membrane potential

In rMTC cells, the membrane potential depended on the extracellular Ca^{2+} concentration. With 1.2 mM external Ca^{2+}, the resting potential was –46.1 ± 1.7 mV (n = 58), and about a third of C-cells spontaneously fired action potentials. On acute rises of the extracellular Ca^{2+} from 1.2 to 1.8 mM, action potentials were induced and/or the cells depolarized by about 10 mV (Fig 1A). In cells constantly bathed in 1.8 mM Ca^{2+}, the membrane potential was –42.1 ± 2.1 mV (n = 56), and action potentials were seen in up to 70% (not shown). Action potentials were reversibly inhibited by Ca^{2+} channel blockers, such as the dihydropyridine isradipine (Fig 1B). The Ca^{2+} channel agonist Bay K 8644 imitated the effect of raising the external Ca^{2+} concentration. At a concentration of 10^{-6} M, Bay K 8644 reversibly depolarized rMTC cells (Fig 1C). In contrast, the membrane potential of PT-r parathyroid cells was not consistently affected by changing the extracellular Ca^{2+} from 1.2 to 1.8 mM (Fig 1D). However, increasing the extracellular Ca^{2+} to as high as 10 mM depolarized PT-r cells (not shown). Isradipine (Fig 1E) as well as Bay K 8644 (Fig 1F) had no effect on the membrane potential; it remained stable at –42.8 ± 2.8 mV (n = 27). Spontaneous action potentials were never observed in PT-r cells.

Voltage-dependent Ca^{2+} channels

To measure Ca^{2+} channel currents, Na^+ and K^+ currents were blocked by substituting choline-Cl for NaCl in the external solution and CsCl for KCl in both internal and external solutions. Ba^{2+} was used as divalent charge carrier (solution E2). Ba^+ currents through Ca^{2+} channels were elicited by test pulses to various potentials from a holding potential of –80 mV. In rMTC C-cells, the observed voltage-dependent inward currents displayed slow inactivation kinetics (Fig 2). Their amplitudes depended on the concentration of the extracellular divalent charge carrier (14,15). In contrast, PT-r parathyroid cells showed fast inactivating voltage-dependent Ba^{2+} currents (Fig 2). They were max-

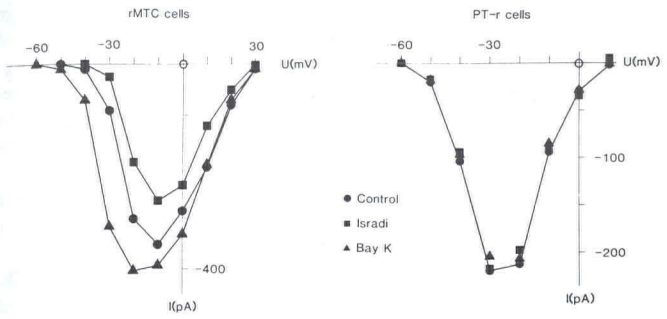

Fig 3—Current-voltage relations of Ca^{2+} channel currents in C-cells and parathyroid cells. Peak Ca^{2+} channel currents elicited by depolarizing test pulses were plotted against the test potentials; the holding potential was –80 mV. Ca^{2+} channel currents in the presence or absence of 500 nM isradipine or 500 nM Bay K 8644 are shown for rMTC-cells on the left panel and for PT-r cells on the right panel. External solution E2, internal solution I2.

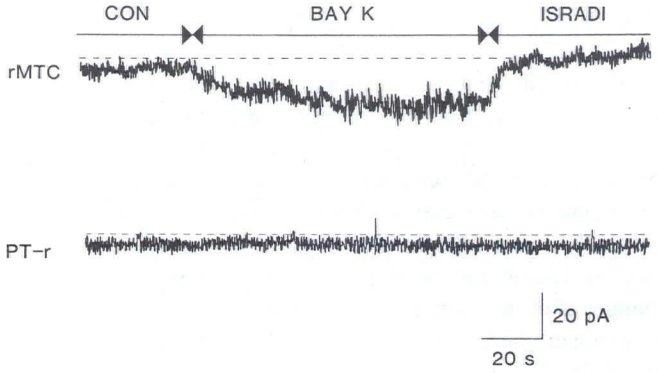

Fig 4—Effects of Bay K 8644 and isradipine on voltage-dependent Ca^{2+} channels in the steady state both in C-cells and parathyroid cells. rMTC (top) and PT-r cells (bottom) were voltage-clamped at a potential of –40 mV for 2 minutes before recording was begun. After the control phase (CON), 500 nM Bay K 8644 followed by 10^{-6} M isradipine was added as indicated. Solutions: pipette solution I2 supplemented with 0.1 to 0.3 mg/mL nystatin; external solution E2. The respective zero current level is indicated by the broken line. No correction was made for leakage.

imal at around –30 mV; at this potential, the mean amplitude amounted to 194.8 ± 36.7 pA (n = 29). The current-voltage relationship (IV curve) revealed an apparent threshold at –60 mV and an apparent reversal at about +10 mV (Fig 3, right panel). Moreover, the depolarization-induced Ca^{2+} channel currents of PT-r cells were neither significantly affected by the (dihydropyridine) Ca^{2+} channel stimulator Bay K 8644 nor by the Ca^{2+} channel blocker isradipine. In contrast to PT-r cells, Bay K 8644 stimulated and isradipine inhibited the depolarization-induced Ca^{2+} channel current of C-cells (Fig 3, left panel). The effects of Bay K 8644 and isradipine on voltage-dependent Ca^{2+} channels also occurred under steady state conditions. The rMTC C-cells and PT-r cells were voltage-clamped close to their resting potentials (–40 mV). Similarly, as reported for GH pituitary cells (16), the steady state inward current through voltage-dependent Ca^{2+} channels of rMTC cells was stimulated by Bay K 8644, suppressed by isradipine (Fig 4, upper panel), and depended on the concentration of divalent cation (14). In contrast, PT-r parathyroid cells exhibited no dihydropyridine-sensitive steady state conductance (Fig 4, lower panel), and neither did rises of the extracellular Ba^{2+} concentration elicit a steady state inward current in PT-r cells (not shown).

Discussion

In C-cells, changes of the extracellular Ca^{2+} cause corresponding changes of the cytosolic free Ca^{2+} concentration which in turn regulates CT secretion (4-6). In the present study, we show that the coupling of the extracellular to the intracellular Ca^{2+} concentration is accomplished by steady state Ca^{2+} influx through noninactivating, dihydropyridine-sensitive, high-threshold Ca^{2+} channels. Agents that block the Ca^{2+} influx cause a fall in the cytosolic Ca^{2+} concentration and inhibit CT secretion. Conversely, rises of the extracellular Ca^{2+} concentration and agents that stimulate the steady state Ca^{2+} influx increase both the cytosolic Ca^{2+} and CT release (3,4,8). The dependency of the Ca^{2+} influx on the extracellular Ca^{2+} concentration is potentiated by the concomitant changes of the membrane potential. Thus, decreases of the external Ca^{2+} hyperpolarize the plasma membrane (17) and thereby reduce the Ca^{2+} current, whereas increases of the external Ca^{2+} depolarize the cellular membrane and increase the current through the voltage-gated Ca^{2+} channels. This particular Ca^{2+} sensitivity of the membrane potential of C-cells most likely results from a sensitive balance of various ionic conductances, above all Ca^{2+}-dependent K^+ channels and the described Ca^{2+} channels.

Stepwise increases in the extracellular Ca^{2+} concentration produce increments in the cytosolic Ca^{2+} and decrements in PTH secretion from parathyroid cells. This inverse relationship between the cytosolic Ca^{2+} concentration and PTH secretion suggests an anomalous stimulus-secretion coupling mechanism (2). However, a doubt exists about a simple association between changes of the cytosolic Ca^{2+} and changes of PTH secretion from parathyroid cells (8,18). Furthermore, voltage-dependent, dihydropyridine-sensitive Ca^{2+} channels are unlikely to play a role in the Ca^{2+} sensitivity of parathyroid cells. Thus, depolarizing concentrations of extracellular K^+, rather than increase, even depress the cytosolic Ca^{2+} and, instead of decreasing, in fact stimulate PTH secretion (2,8,18,19). Our electrophysiological studies similarly argue against dihydropyridine-sensitive Ca^{2+} channels as the mechanism by which extracellular Ca^{2+} could cause increases in Ca^{2+} influx in parathyroid cells. Under voltage-clamp conditions, PT-r parathyroid cells exclusively exhibited fast inactivating, dihydropyridine-insensitive Ca^{2+} currents. Increases of the concentration of the external divalent charge carrier Ba^{2+} failed to cause a steady state Ba^{2+} influx. Neither did changes in the extracellular Ca^{2+} (within the physiological range) cause any consistent effects on the membrane potential.

Thus, the mechanisms underlying the Ca^{2+} sensitivity of parathyroid cells remain unclear. Recently, a cell surface "Ca^{2+} receptor" or "divalent cation receptor" has been proposed as sensor of the extracellular Ca^{2+} concentration in parathyroid cells (2,18,20). An alternative possibility to consider in normal parathyroid cells is the existence of nonselective cation channels. Since nonselective cation channels allow increased Na^+ and Ca^{2+} influx on membrane hyperpolarization and reduced ion influx on membrane depolarization (21), they could couple changes of the membrane potential induced by major changes of the extracellular Ca^{2+} concentration (22,23) to changes in the intracellular Ca^{2+} concentration and could thereby regulate PTH secretion.

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