IONIC CURRENTS IN ADRENAL CHROMAFFIN CELLS FROM RATTUS RATTUS IN PRIMARY CULTURE

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The first objective of this project was to define culture procedures for rat chromaffin cells, survey their ionic currents, and define ionic conditions which might be suitable for measurement of depolarization-activated secretion.

Primary cultures of rat chromaffin cells were prepared using a modification of a method previously used for bovine chromaffin cells (L. Gandia, A.G. Garcia, & M. Morad, J. Physiol. 470:55-72, 1993). Briefly, adrenal glands were removed aseptically from 4 anaesthetized rats (350-400 g). Each medulla was dissected free of the cortex, rinsed, cut into fragments and cells were dispersed with a solution containing collagenase and bovine albumin. Remaining tissue fragments were removed by filtration through a 72 μm nylon mesh. The cells were rinsed twice using centrifugation (15 min, 200g), washing and resuspension. Density fractionation with Urografin (Schering AG) was used to remove non chromaffin cells. After centrifugation (20 min, 6000g, 22 °C) the cells at the 15%/7.5% interface were plated onto collagen treated cover slips or into thin-bottom disposable perfusion chambers. The cells were maintained in culture (Dulbecco's modified Eagle's medium, SIGMA D-5536, with 10% fetal bovine serum , SIGMA F-0643, and penicillin and streptomycin, SIGMA P-3539) up to one week in a humid atmosphere containing 5% CO_2 at 37 °C.

The cultures typically contained a mixture of cells, spherical or spreading, with or without rough granulation, all adhering to the bottom. Cells used in voltage clamp studies were spherical (20 μm in diameter) and appeared transparent under moderate magnification (x25 objective). Higher magnification (x63, NA 1.3) revealed a fine granulation. Sectioning with confocal reflectance microscopy shows the nucleus with nearby structures radiating towards the cell surface.

The ionic currents were measured at room temperature in the whole cell clamp configuration using patch pipettes with 2 to 5 ΩM resistance filled with a Cs^+- based dialyzing solutions (137 mM CsCl, 5 mM MgATP, 0.01 mM cAMP, 10 mM HEPES at pH 7.2) with different amounts of Ca^{2+}-buffer. When the internal Ca^{2+} concentration was buffered with 5 mM EGTA we observed only two inward current components. The larger (about 1 nA peak) was identified as a Na^+-current based on the potential dependencies of its activation and inactivation, its almost complete inactivation within 5 ms, and its complete block by TTX. The smaller inward current component (30 to 300 pA peak) was identified as Ca^{2+}-current and had the following properties: It showed little inactivation (<20%) on a time scale of 100 ms, activated at potentials more positive than the Na^+-current, was subject to relatively rapid run-down, was blocked by 100 μM Cd^{2+}, was insensitive to 10 μM isoproterenol, and doubled in magnitude when the

extracellular Ca^{2+} was increased from 2 to 10 mM. With less cytosolic Ca^{2+} buffering (0.1 mM BAPTA) an outward current component was observed which, on repolarization to -60 mV had a long-lasting inward tail current (Figure 1A). This current resembled the Ca^{2+}-activated K^+-current described by Neely and Lingle (J. Physiol. 453:97-131, 1992). It should be noticed, however, that the present current is relatively small, is carried in the outward direction probably by Cs^+, rather than by K^+, and requires extracellular K^+ for this efflux to take place (Figure 1B). The estimated selectivity ratio pK/pCs was about 7/1 even though Neely and Lingle found a high degree of K^+ selectivity in Na^+-containing solution. We did not attempt to analyze the outward current in terms of components with different time-, voltage- and drug-dependence, nor did we examine the presence of different types (L, N, and P) of Ca^{2+} currents.

Our results suggest that the cultured rat chromaffin cells expressed Ca^{2+} current which could be enhanced by increasing the extracellular Ca^{2+} concentration. The Ca^{2+}-activated outward current measured with Cs^+ as charge carrier may provide a convenient, indirect way of detecting the onset of rise in Ca^{2+} in the absence of Ca^{2+}-sensitive dyes.

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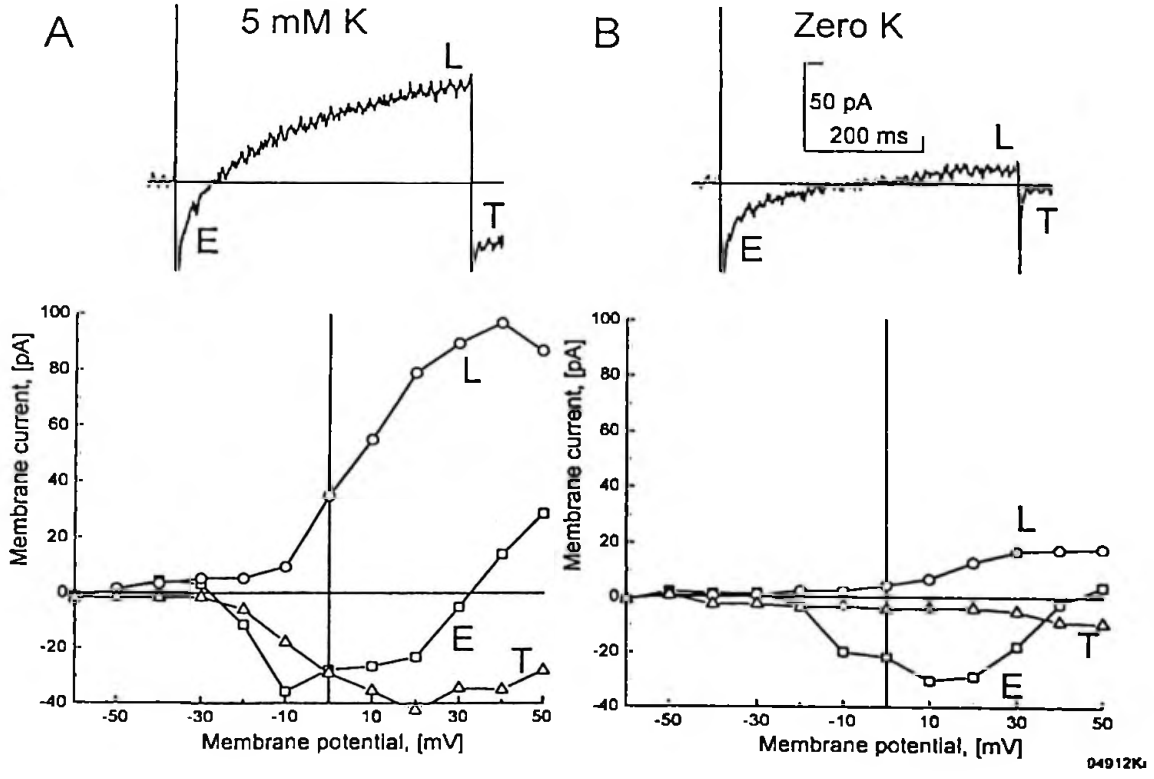

Figure 1. Voltage dependence membrane current in the presence and absence of 5mM extracellular K^+. The Ca^{2+} current (E, squares) was measured as the average from 10 to 20 ms after depolarization and was insensitive to extracellular K^+. The time dependent development of the late outward current (L, circles) and of the tail current (T, triangles) was strongly suppressed by removal of extracellular K^+ (Cf. panels A and B).