Transient Ca\(^{2+}\)-channel current characterized by a low-threshold voltage in zona glomerulosa cells of rat adrenal cortex

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Abstract. Voltage-gated Ca\(^{2+}\)-current was identified in single isolated cells of the zona glomerulosa of adrenal cortex and its properties were studied by the "tight-seal" whole cell recording technique. The Ca\(^{2+}\)-channel current was dissected from the net current by dialyzing the cells with CsCl. The identified Ca\(^{2+}\)-current was found to be activated by a relatively small depolarization only when the cells were held at a large negative holding potential, but it was inactivated within 10–30 ms. The time course of activation and inactivation was voltage-dependent and became faster when the amplitude of depolarization was increased. The transmembrane potential of the glomerulosa cells was highly sensitive to [K\(^+\)], the slope of the potential change per tenfold change in [K\(^+\)], being 48 mV. An increase in [K\(^+\)] from 4.7 to 10 mM induce a membrane depolarization by 15 mV, since an elevation of K\(^+\) concentration may cause a cell membrane depolarization. The present study has aimed at demonstration of the presence of such a Ca\(^{2+}\)-channel and clarifying the properties of the channel of this particular type of endocrine cells.

There is accumulating evidence for diversity of Ca\(^{2+}\)-channels in various kinds of cells (Hagiwara 1983, Nowycky et al. 1985). As for endocrine cells, for examples, chromaffin cells and clonal cells of anterior pituitary tumor have been shown to possess a voltage-gated Ca\(^{2+}\)-channel, characterized by activation by a relatively large depolarization and slowly developing inactivation (Fenwick et al. 1982; Hagiwara and Ohmori 1982). On the other hand, a different type of voltage-gated Ca\(^{2+}\)-channels have been reported for cultured anterior pituitary cells which is activated by a relatively small depolarization at large negative holding potentials and characterized by rapidly developing inactivation (Armstrong and Matteson 1985). The results of the present study show that the adrenal glomerulosa cells from rat possess the latter type of Ca\(^{2+}\)-channels and the relationship between the membrane potential and external K\(^+\) concentration may well explain the activation of this Ca\(^{2+}\)-channel by a small increase in external K\(^+\) concentration.

Key words: Ca\(^{2+}\)-current — Glomerulosa cell of adrenal cortex — Whole-cell recording — K\(^+\)-stimulation

Introduction

Studies of the mechanisms underlying Ca\(^{2+}\) permeability appears to be a prerequisite for the understanding of the secretary process in various secretory cells (Petersen 1980). For the cells of zona glomerulosa of adrenal cortex, angiotensin II, adrenocorticotropic, and elevation of the external K\(^+\) concentration are known to be three major stimulants for aldosterone secretion (Saruta et al. 1972; Aguilera and Catt 1979). Recent studies using radioactive Ca\(^{2+}\) have shown that all these stimulants cause an increase in Ca\(^{2+}\) influx across the cell membrane (Kojima et al. 1985). In the case of K\(^+\) stimulation, the increase in intracellular Ca\(^{2+}\) which is needed for aldosterone secretion is entirely dependent on the extracellular source (Kojima et al. 1984). This suggests that there exists a voltage dependent Ca\(^{2+}\)-channel in the cell membrane of the zona glomerulosa cells since an elevation of K\(^+\) concentration may cause a cell membrane depolarization. The present study has aimed at demonstration of the presence of such a Ca\(^{2+}\)-channel and clarifying the properties of the channel of this particular type of endocrine cells.

Materials and methods

Cell preparations. Single isolated glomerulosa cells were prepared from adrenal capsular tissue of male Sprague-Dowley rats (150–200 g). After decapitation of the animal adrenal glands were quickly dissected, rinsed with normal Ringer’s solution, and all fat was removed. The capsular tissue, which was used for obtaining single glomerulosa cells, was carefully stripped from the glands. The fragments of capsular tissue were then incubated in normal Ringer’s solution containing 200 μ/ml collagenase (Sankyo, Tokyo, Japan) for up to 20 min at 37°C. At the end of the digestion period there were undigested tissue debris, clusters of cells as well as single isolated cells. After the digestion, we noticed that the population of zona fasciculata cells was small, even the thickness of this layer is largest. Under an inverted microscope at a magnification of ×800, cells of zona fasciculata, which locates in between zona glomerulosa and zona reticulata, can be easily differentiated from others by the presence of typical lipid droplets in the cytoplasm. This seems to us to indicate that the capsule was stripped with the cells existing in the outermost layer, zona glomerulosa. Thus, the cells we used should be derived from zona glomerulosa and possible encounter with those from zona...
potentials between these solutions was 3 - 5 mV, the pipette potential as zero-current potential was between -65 and -70 mV.

Whole-cell current clamp recording was performed to estimate the resting potential, and to measure the spike responses to step-like changes in current in single glomerulosa cells. The estimate of the resting membrane potential as zero-current potential was between -80 to -40 mV when measured in the voltages ranging from -60 to -60 mV and the spikes never exceeded 0 mV. Neither the after-polarization nor the anodal break response were observed.

Figure 1B shows current responses to step-wise changes in voltage and their current-voltage relationship taken under the quasi-physiological ionic gradients. In these experiments, initial inward currents as well as following outward currents were elicited by the voltages more positive than -60 mV whereas depolarizing currents induce voltage spikes in all-or-none fashion whereas hyperpolarizing steps can only induce passive voltage changes. The threshold potential of the spikes was about -60 mV and the spikes never exceeded 0 mV.

Results

Whole-cell current- and voltage-clamp recording

Whole-cell current clamp recording was performed to estimate the resting potential, and to measure the spike responses to step-like changes in current in single glomerulosa cells. The estimate of the resting membrane potential as zero-current potential was between -65 and -80 mV (-73 ± 5.1 mV, n = 7, or about -70 mV if corrected for the liquid-junction potential), under a quasi-physiological ionic gradients across the membrane: That is, the cells were dialyzed with the K-solution and immersed in the normal solution. Figure 1A shows superimposed voltage traces in response to step-wise changes in current and their current-voltage relationship, in which depolarizing current-steps induce voltage spikes in all-or-none fashion whereas hyperpolarizing steps can only induce passive voltage changes. The threshold potential of the spikes was about -60 mV and the spikes never exceeded 0 mV. Neither the after-polarization nor the anodal break response were observed.

Figure 1B shows current responses to step-wise changes in voltage and their current-voltage relationship taken under the quasi-physiological ionic gradients. In these experiments, initial inward currents as well as following outward currents were elicited by the voltages more positive than -60 mV. In later part of the present paper, we will describe the properties of this inward current "dissected" from the net current using Cs-loaded cells.

Input resistance in the whole-cell variation varied from 26.3 GΩ in the membrane potential ranged between -100 and -205 mV, and that of 5.4 GΩ in between -78 and -35 mV (this value was the upper limit for the input resistance measured in the whole-cell variation). Filled circles show the sizes of electrotonic component, whereas open circles show those of spikes. B Superimposed voltage-clamp records and their I/V-relationship. The holding potential was -80 mV. Is-s and Ipeak denote the current measured at the end of voltage-pulses and at the peak, respectively.