SECRETION IN ADRENAL CHROMAFFIN CELLS FROM RATTUS RATTUS IS GATED BY L-TYPE Ca^{2+} CHANNEL

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Individual mammalian chromaffin cells are known to express Ca^{2+} channels of L, N, P and Q type. There is now evidence that the L-type Ca^{2+} channel in bovine (Artalejo, Adams, and Fox, Nature, 367:72, 1994) and cat (Lopez, Albillos, de la Fuente, Borges, Gandia, Carbone, Garcia, and Artalejo, Pflugers Arch., 427:348, 1994) chromaffin cells controls secretion to a larger extent (>80%) than might be expected from its magnitude (about 50% of total Ca^{2+} current). We have examined this phenomenon in cultured rat chromaffin cells (Fan, Cleemann, Lara, Gandia, and Morad, present volume of the Bull. MDIBL, 1995) using carbon fiber electrodes to measure the secretion, both during and after voltage-clamp depolarizations, with high sensitivity and temporal resolution.

The carbon fiber electrodes were prepared from single filaments (6-8 μm in diameter, Thornel, Greenville, SC 29602) insulated with polyethylene tubing (300μm inner diameter) which was heated in two stages first being stretched and collaped around the fiber. The fiber was then cut in two, and each end reheated so that the plastic covering receded from its tip leaving only a very thin coating over a length of a few hundred microns. Before the use of the carbon fiber electrode, its tip was broken, often repeatedly, until the break appeared as a single clean surface under the microscope and the current produced by a sawtooth potential (-700 mV to +900 mV, 10 Hz) resembled that of an ideal polarizable electrode. Suitable carbon fiber electrodes had low capacitance and resistance but strong rectification in both directions and, in characteristic potential regions, currents indicative of electrode processes such as hydrolysis of water and oxidation of catacholamines (about 200 pA with a test solution containing 0.1 mM epinephrine). During measurements of secretion the carbon fiber was used in the amperometric mode (biased at +600 mV) and was pushed gently against a chromaffin cell from one side. The cell was then approached with a glass pipette from the other side, and giga-seal and break-in were established. The carbon fiber electrode and the patch electrode were attached to separate DAGAN (model 8900) amplifiers, but were controlled with a single IBM compatible personal computer using the pCLAMP program. A junction box was used to switch the single programmable command potential and steady bias potentials between the carbon fiber electrode and the patch clamp electrode.

The voltage and time dependence of membrane current and secretion was measured as shown in Fig. 1. The secretory response was seen as current spikes (max 50 pA in amplitude and about 20 ms in duration) thought to represent exocytosis of individual secretory granules. Voltage clamp procedures were always repeated several times since each depolarization typically produced only a few secretory events. The figure shows that the average secretory response (bottom) has basically the same bell shaped voltage dependence as the Ca^{2+} current

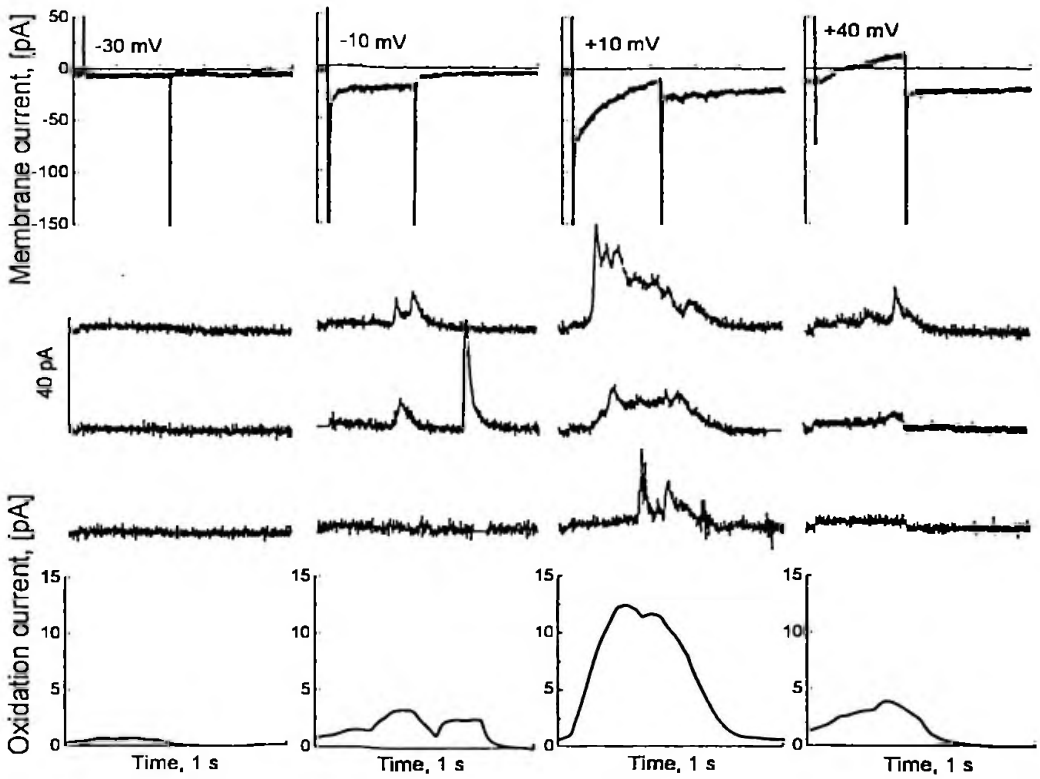

Figure 1. Membrane current and secretion in a rat chromaffin cell clamped to different membrane potentials (different columns). From top to bottom the traces are the membrane current, 3 secretory responses recorded during 3 series of voltage clamps, and, at the bottom, the averaged and filtered (200 msec window) secretory response. The holding potential was -70 mV and the duration of the clamp pulses 400 ms. The external solution contained 137 mM NaCl, 5 mM KCl, 10 mM $CaCl_2$, 10 mM glucose and 10 mM HEPES at pH 7.4 and the internal solution 137 mM CsCl, 5 mM MgATP, 0.01 mM cAMP, 0.1 mM BAPTA and 10 mM HEPES at pH 7.2. The resting interval between depolarizations was 20 sec.

(Top). This impression was substantiated by plotting the voltage relations of both. The long lasting current tails recorded following repolarization of each voltage clamp pulse reflect the properties of a Ca^{2+}-activated K^+ channel. Using these current tails as indicators for the intracellular Ca^{2+} activity we found basically the same bell-shaped voltage dependence as mentioned above. In other experiments the voltage dependence of the secretory response was measured with 10 or more consecutive depolarizations to the same potential. During a 400 ms depolarization to +10 mV the secretory rate reached a steady level with a time constant of 150 to 200 ms. Following repolarization the secretion declined to zero with a similar time constant. It may be noticed that this decline is faster than the decline of the current tails or of the intracellular Ca^{2+} transients measured by others. Thus it appears that secretion exhibits a delay of 150 to 200 ms, but that it otherwise follows the Ca^{2+} current more closely than the global change in

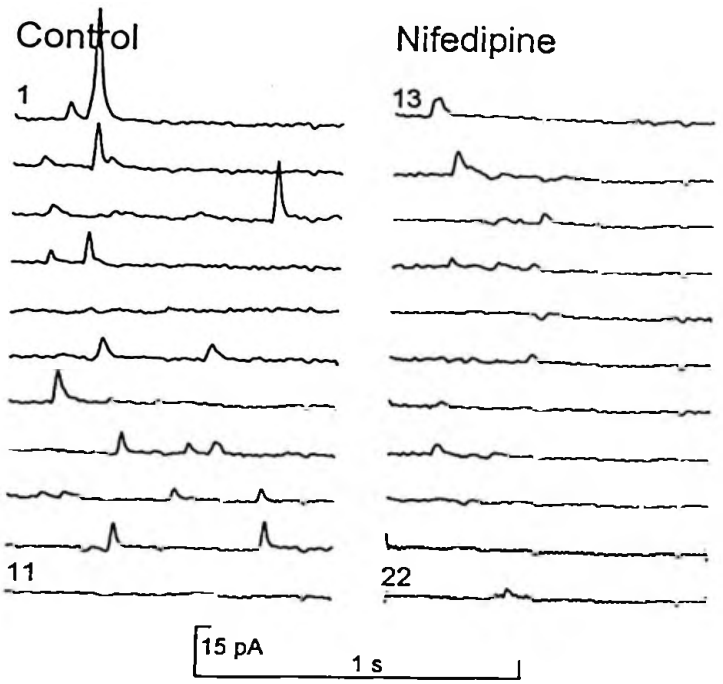

Figure 2. Block of secretion by 5 μM nifedipine. A train of 22 secretory responses straddling the addition of nifedipine. The holding potential was -70 mV and the depolarizations to +10 mV lasted 400 ms.

cytoplasmic Ca^{2+}. A possible explanation for this finding is that [Ca^{2+}] builds up near the inner openings of Ca^{2+} channels resulting in exocytosis of docked secretory vesicles in the immediate vicinity.

Since this scheme allows a differential role for different types of Ca^{2+} channels we measured how secretion was modified when 40 to 50% of the total Ca^{2+} current was blocked with 5 μM nifedipine, a selective blocker of the L-type current. Figure 2 shows results from an experiment where 11 consecutive depolarizations were applied both before and after the addition of the drug. Comparison of the left and right side of the figure shows that secretion was suppressed by about 80% by nifedipine. Washout of the nifedipine (not shown) resulted in partial recovery of Ca^{2+} current and secretion.

Our results are consistent with the idea that exocytosis of secretory granules is controlled primarily by the L-type Ca^{2+} channel. They are therefore in agreement with the results obtained from bovine and cat chromaffin cells. There is some indication, however, that the effect of nifedipine on Ca^{2+} current is faster than its effect on secretion. The Ca^{2+} current reached the new reduced level from one voltage clamp pulse to the next, 20 sec later. Yet, the secretion was still noticeable during the next two depolarizations (Right panel of Fig. 2, traces #12 and #13). This suggests that the L-type Ca^{2+} channel might be more directly involved in the Ca^{2+}-dependent docking of secretory vesicles (Neher and Zucker, Neuron, 10:21, 1993) than in the actual exocytosis of release-ready vesicles. The test of this possibility will require more extensive studies with rapid application and removal of blockers of different types of Ca^{2+} channels.

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