the chloride secretion rate versus chloride concentration is depicted in Figure 7. The slope of this relation is 1.0, $p < 0.001$, indicating that in the presence of nitrate only one chloride interacts with the transport system. Consistent with this inference, a Lineweaver-Burke plot of the rate of chloride secretion versus the concentration of chloride in the perfusate is linear (Figure 8). These findings suggest also that nitrate can replace chloride at one of its sites of interaction with the contransport carrier.

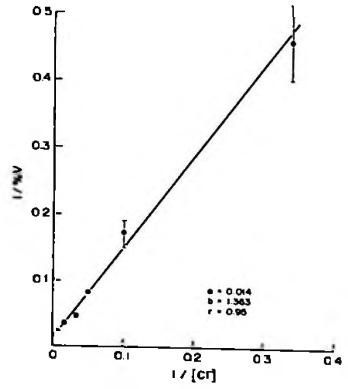

Figure 7.--Hill plot of the relation between chloride secretion and chloride concentration shown in Figure 6. The Hill coefficient is 1.0 indicating that in the presence of nitrate chloride interacts with only one site in the chloride transport sytem.

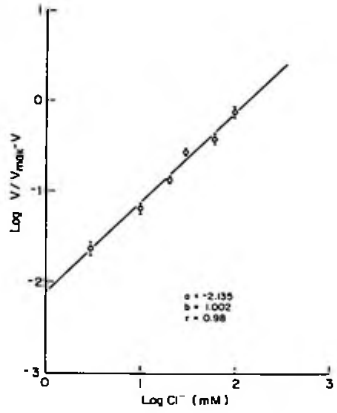

Figure 8.--Lineweaver-Burke plot of the chloride secretion data shown in Figure 6. The relation is linear. A Km of 100 μM for chloride can be calculated suggesting that nitrate is interacting with the high affinity site.

These findings provide strong support for the simultaneous interaction of $1Na^+$ and $2Cl^-$ with the rate-limiting co-transport carrier in stimulated secretion by the rectal gland. A similar mechanism, presumably involving $1\ Na^+$, $1K^+$ and $2Cl^-$, probably is present in other cells, including flounder intestinal mucosa, Erlich ascites tumor, avian erythrocytes, and the mammalian thick ascending limb. Unexpectedly, nitrate, but not gluconate, appears to have a limited ability to substitute for chloride on one of its carrier sites, and this may prove to be the case in other chloride transporting systems as well.

ANTIPYRYLAZO III MEASUREMENTS OF EXTRACELLULAR Ca^{2+} DEPLETION IN VOLTAGE CLAMPED FROG VENTRICULAR MUSCLE

Lars Cleemann, JoAnna S. Choo and Martin Morad, University of Pennsylvania, Department of Physiology, Philadelphia, Pa.

Ca^{2+} for activation of tension in cardiac muscle cells is thought to depend in part on transport of Ca^{2+} across the membrane and in part on the release of Ca^{2+} from intracellular pools. To assess the role of extracellular Ca^{2+} in immediate activation of tension we decided to investigate whether Ca^{2+} enters the cell in sufficient quantity to allow measurement of its depletion from the extracellular space. For this purpose we have developed an optical technique which utilizes the color change produced by antipyrylazo III, an impermenant Ca-sensitive dye. Preliminary results show that a Ca depletion signal can be measured after careful elimination of contraction artifacts. This signal has a wavelength dependency predicted by spectrophotometric measurements of free dye in Ca^{2+}-containing solutions. The Ca^{2+}-depeltion signal appears to include a component which might reflect the slow inward current. It was concluded that the development of the optical technique to measure Ca^{2+} movement across the membrane provides data on the kinetics of Ca^{2+} movement across the membrane not easily obtained with much slower Ca^{2+}-sensitive electrodes.

The experimental set-up was a modified single sucrose gap voltage clamp set-up which allowed simultaneous measurements of light transmission at three different wavelengths. Frog ventricular strips were mounted, as usual, with one end attached to a force transducer and the other end extending through the sucrose gap. Then the strips were gently squeezed between the moveable transparent walls of the perfusion chamber to eliminate gross movements of the preparation. Further reduction of contraction artifacts was achieved by stretching the strip and by lowering the Ca activity of the perfusate to 0.2 mM. At this value antipyrylazo III is a suitable Ca indicator dye.

Panels B, C and D of Figure 1 show records obtained under these conditions. Each panel shows the action

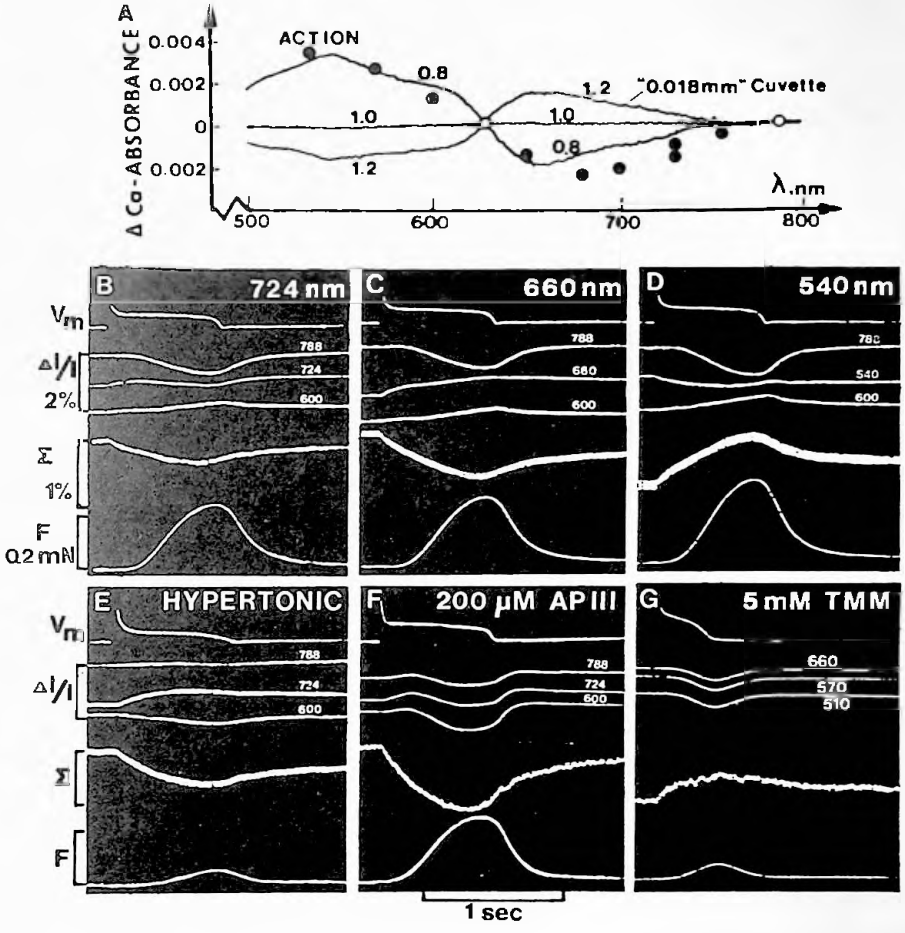

Figure 1.--Measurements of extracellular Ca depletion under various conditions. Panel A shows the action spectrum. The Ca depletion signal measured at different wavelengths (points) is compared to the Ca induced difference spectrum measured with a spectrophotometer. The action spectrum is obtained from measurements at different wavelengths similar to those shown in the panels B, C and D. In panel E the twitch is reduced by a hypertonic perfusate but the dye and Ca concentrations are as in panels B, C and D(2 mM antipyrylazo and 1 mM Ca total, $[Ca]_o \cong 0.2$ mM). In panel F the concentration of antipyrylazo is reduced (0.2 mM antipyrylazo and 0.25 mM Ca total, $[Ca]_o \cong 0.2$ mM) and in panel B the Ca sensitive dye was tetramethylmurexide (5 mM tetramethylmurexide and 2 mM Ca total, $[Ca]_o \cong 1$ mM0. From top to bottom the panels B through G show the membrane potential measured through the sucrose gap (V_m), the change in the light intensities measured at three wavelengths ($\Delta I/I$), the Ca depletion signal (Σ), and the twitch force (F). The inversion of the Ca depletion signal in the panels D and F are predicted by the spectrophotometric measurements (Cf. panel A). The scales in the panels E, F and G are ($\Delta I/I$:1%, 1% and 5%; Σ: 0.5%, 0.2% and 0.2%) and F: 0.2 mN, 0.2 mN and 1 mN.

potential at the top (V_m), the isometric twitch force at the bottom (F) and in between the optical signals. The three light intensities ($\Delta I/I$) are measured at 788 nm where the dye is transparent, at 600 nm which is close to the isosbestic

point and at a third wave length selected from the regions of the spectrum where a Ca signal may be expected (724 nm in panel B, 660 nm in panel C and 540 nm in panel D). A weighted average of the contraction artifacts at 788 nm and 600 nm is subtracted from the signal at the third wave length in order to remove the contraction artifact which is superimposed on the Ca signal. The weights are calculated from the measured absorption spectra in wuch a way that the result (Σ) should be insensitive to small movements of either the strip or the dye-filled extracellular spaces. Comparison of panels B, C and D shows that this protocol gives Ca depletion signals with a time course which is independent of wave length. The amplitude of the signal varies as predicted from spectrophotometric measurements of the Ca induced difference spectrum of the perfusate (Panel A). Thus the signal is larger at 660 nm than at 724 nm and it is reversed at 540 nm on the other side of the isosbestic point.

The lower panels in Figure 1 show Ca depletion signals obtained under different conditions. In panel (E) contraction was suppressed by hypertonic solution. A flat trace at 788 nm shows the absence of a significant contraction artifact. The major contribution to the depletion signal (Σ) seems to come from the increased light intensity at 724 nm during the action potential. The small downward deflection at 600 nm is consistent with this wavelength, being a little below the isosbestic point. Panels F and G show records from experiments where the concentration of antipyrylazo was reduced or where the Ca sensitive dye was tetramethylmurexide. The time course of the Ca depletion signal was similar to that of previous panels even though contraction artifacts played a more dominant role. Experiments with and without dye and with Ca insensitive dyes show that the weighing procedure reduces contraction artifacts by a factor of 10 to 20. This finding suggests that reliable measurements of Ca depletion can be performed only when the light intensity at a specific weavlength includes a Ca signal comparable to or larger than the superimposed contraction artifacts.

Figure 1 shows representative tracings of the time course of the Ca depletion signal. The rate of Ca depletion was maximum immediately after the upstroke of the action potential but prior to the onset of twitch, and it decreased slowly during the plateau of the action potential. Peak Ca^{2+} -depletion occurred toward the end of the action potential but before the peak of the twitch. After the action potential the Ca-depletion signal returned to baseline values so slowly that depletion was still noticeable long after relaxation was complete. Calibration against spectrophotometric measurements showed that 0.01 to 0.2 nM Ca is removed from the extracellular space during each beat.

The effects of rapid beating, low $(Na)_o$, digitalis, epinephrine and voltage clamp pulses on extracellular Ca depletion were explored. Figure 2 shows how the extracellular Ca depletion was enhanced when the epinephrine was

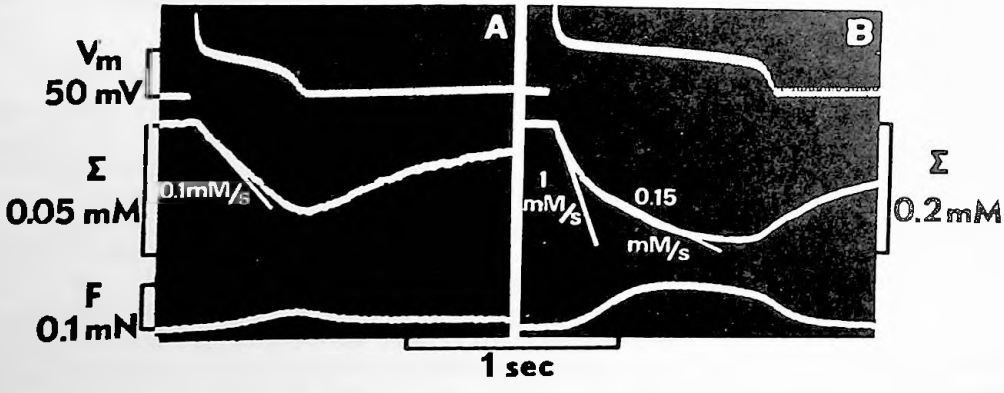

Figure 2.--Measurement of Ca depletion in the absence (Panel A) and presence (Panel B) of epinephrine. From top to bottom each panel shows the action potential measured through the sucrose gap (V_m), the Ca depletion signal in mM measured at 724 nm (Σ), and the isometric twitch force (F). Note that Σ has different scales in the two panels.

used to prolong the action potential and potentiate the twitch. During the early part of the action potential the rate of Ca depletion increased from 0.1 mM/sec to 1 mM/sec in the presence of epinephrine.

Figure 3 demonstrates the feasibility of combining voltage clamp procedures with optical measurements of

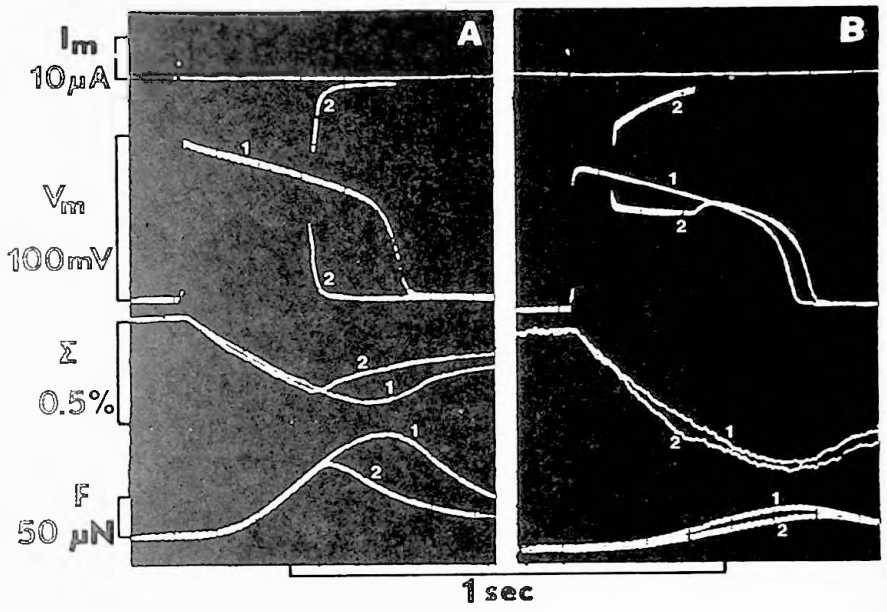

Figure 3.--Voltage clamp records. Each panel shows the membrane current (I_m), the membrane potential (V_m), the Ca depletion signal (Σ), and the isometric force (F). Panel A shows that the Ca depletion is terminated shortly after the membrane potential is clamped back to the resting potential. Panel B shows that interruption of the plateau with a clamp pulse to -20 mV is accompanied by inward current and increased Ca depletion.

extracellular Ca depletion. In panel A the action potential was terminated prematurely by clamping the membrane back to the resting potential. Note that this procedure terminated the Ca-depletion signal almost immediately while the force response lagged behind about 50 msec. In panel B the plateau of the action potential was interrupted by a 200 msec clamp pulse to -20 mV. The clamp pulse was accompanied by inward current and an increased rate of Ca-depletion. These findings suggest that measurements of extracellular Ca-depletion may be used to examine to what extent the measured membrane currents are carried by Ca ions.

Our results show that this optical method can be used to measure extracellular Ca-depletion in frog ventricular strips. Contraction artifacts present a serious but not insurmountable problem, and the great advantage gained from the high time-resolution makes this technique the method of choice.

The findings reported here suggest that the extracellular Ca-depletion is in part due to the influx of Ca through the "slow inward" channel. This is supported by the findings that the rate of Ca-depletion is decreased during the time course of the action potential, and by the observation that epinephrine increased the rate of Ca-depletion. More extensive voltage clamp experiments are required to substantiate a correlation between the slow inward current and the extracellular Ca-depletion. Such experiments might also reveal the properties of other sarcolemmal Ca transport mechanisms such as Na/Ca exchange. This work was supported by NIH Grants HL16152 and HL29329.

SODIUM AND POTASSIUM DEPENDENT CHLORIDE UPTAKE BY RECTAL GLAND PLASMA MEMBRANE VESICLES

J. Hannafin, E. Kinne-Saffran, B. Vickermann and R. Kinne, Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY and Max Planck Institut für Systemphysiologie, Dortmund, West Germany

Introduction

The rectal gland of the dogfish, Squalus acanthias has been studied extensively during the last decade as a