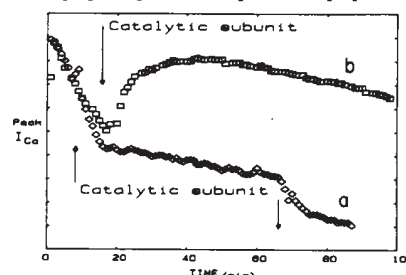


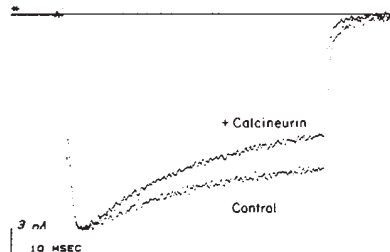
T-Pos114 LEUPEPTIN, AN INHIBITOR OF Ca-DEPENDENT PROTEASES, RETARDS THE KINASE-IRREVERSIBLE, Ca-DEPENDENT LOSS OF CALCIUM CURRENT IN PERFUSED SNAIL NEURONS. J. Chad and R. Eckert, Department of Biology, UCLA, Los Angeles, CA 90024.

Perfusion of *Helix* neurons is accompanied by a progressive 'wash out' of calcium current, I_{Ca} . This loss is slowed or partially reversed by means that enhance cAMP-dependent phosphorylation (a in fig.; Chad and Eckert, 1984, *Neurosci. Abstr.* 10:866). However, as Ca-mediated inactivation and wash out proceed, the effectiveness of phosphorylating agents in promoting recovery of I_{Ca} diminishes, suggesting irreversible loss of channels. Wash out is slowed by maneuvers that reduce Ca_i and Ca-dependent inactivation. This suggested a Ca-dependent degradation of the Ca channel that may occur preferentially from its inactivated state, and this was tested under voltage clamp (100ms pulses to +10 mV from -40 mV) with the tripeptidyl aldehyde leupeptin (Aoyagi *et al.*, 1969, *J. Antibiotics* 22:558), an inhibitor of Ca-dependent proteases (Yoshimura *et al.*, 1983, *J. Biol. Chem.* 258:8883). Addition of 100 μ M leupeptin to the perfusate had little effect on I_{Ca} amplitude, inactivation, or rate of washout in the absence of phosphorylating additives. However, irreversible loss of I_{Ca} was greatly reduced by perfusion with leupeptin (b in fig.), for introduction of catalytic subunit of cAMP-dependent PK plus ATP-Mg effectively restored I_{Ca} to nearly its original amplitude. The effect of leupeptin suggests that an endogenous Ca-dependent protease may account for the irreversible loss of Ca channel activity in perfused neurons. USPHS NS 8364.



T-Pos115 CALCINEURIN, A CALCIUM-DEPENDENT PHOSPHATASE, ENHANCES Ca-MEDIATED INACTIVATION OF Ca CURRENT IN PERFUSED SNAIL NEURONS. J. Chad and R. Eckert, Department of Biology, UCLA, Los Angeles, CA 90024.

Isolated neurons of *Helix aspersa* were perfused and voltage clamped under conditions that isolate the calcium current, I_{Ca} . Perfusate containing HEPES (100 mM, pH 7.8), aspartate (20 mM), CsOH (~70 mM) TEA-Cl (10 mM), catalytic subunit of cAMP-dependent protein kinase (~25 μ g protein ml^{-1}), ATP-Mg (4 mM) and leupeptin (100 μ M) maintained I_{Ca} with little loss for periods greater than 100 minutes. External Ca was 10 mM and internal was nominally 0.01 μ M, but is predicted to undergo large excursions near Ca channels during Ca entry. Addition of calcineurin (~80 μ g protein ml^{-1}), a Ca-dependent phosphatase (Steward *et al.*, 1982, *FEBS Lett.* 137:80) to the perfusate in the presence of leupeptin had little effect on peak I_{Ca} , but produced a marked increase in rate of Ca-dependent inactivation during 100ms pulses to +10 mV from -40 mV. This is contrary to the behavior predicted from possible increased Ca-buffering capacity due to addition of a Ca-binding protein, and implies that the increased rate of Ca-dependent inactivation is due to the increased enzymatic phosphatase activity. The enhancement of Ca-mediated inactivation by a Ca-dependent phosphatase supports the hypothesis (Eckert and Chad, 1984, *Prog. Biophys. Mol. Biol.*) that a Ca-dependent dephosphorylation is the pivotal step effecting the Ca-mediated inactivation of the Ca channel, with phosphorylation required for returning the channel to an activatable state.



T-Pos116 ROLE OF CYCLIC NUCLEOTIDES IN REGULATION OF SLOW CHANNEL FUNCTION IN VASCULAR SMOOTH MUSCLE. Julia M. Ousterhout and Nick Sperelakis. Dept. of Physiology & Biophysics, Univ. of Cincinnati, Cincinnati, OH 45267.

Cyclic AMP (cAMP) is involved in the regulation of myocardial slow channels, presumably via activation of a cAMP-dependent protein kinase and phosphorylation of the slow channels or an associated regulatory protein. Our laboratory has recently shown that cyclic GMP (cGMP) also modulates the slow inward current in myocardial cells, but in a direction opposite to that of cAMP (Wahler and Sperelakis, 1984). The purpose of this study was to determine whether or not cyclic nucleotides regulate slow channel function in vascular smooth muscle (VSM). Cultured VSM cells were prepared from adult rat aortas by enzyme dispersion (collagenase or trypsin) and reaggregation. Ca^{2+} -dependent action potentials (APs) were elicited by electrical stimulation in the presence of tetraethylammonium (TEA, 5-15 mM). Superfusion of the reaggregates with dibutyryl cAMP was found to depress (0.1 mM) and abolish (0.5-1 mM) the TEA-induced APs. In contrast, in an intact strip preparation of the rabbit pulmonary artery, dibutyryl cAMP (0.1-2 mM) had little or no effect on TEA-induced APs. Superfusion of aortic reaggregates with dibutyryl cGMP (1 mM) or 8-bromo-cGMP (0.1-1 mM) also had little or no effect on TEA-induced APs; i.e., they were not inhibited or potentiated. These results suggest that cAMP, but not cGMP, may regulate the function of the voltage-dependent Ca^{2+} slow channels in rat aortic smooth muscle cells. (This study was supported by NIH Grant HL-31942, Training Grant HL-07382, and Postdoctoral Fellowship HL-06835.)