Th-AM-MinI-1 ELECTROPHYSIOLOGICAL APPROACHES TO ENDOCRINE FUNCTION: A Mini-Symposium.

Organized by Gerry S. Oxford, University of North Carolina, Chapel Hill, NC 27514.

For many years the property of membrane excitability was thought to be the exclusive province of nerve and muscle cells. It has become increasingly clear, however, that non-excitable cells are more the exception than the rule. The discovery of regenerative and often spontaneous electrical activity in endocrine cells has led to many hypotheses for the involvement of membrane permeability and potential changes in processes other than neural transmission and muscle contraction. For example, the Ca-dependence of action potentials and of hormone exocytosis in several cells has prompted analogies between secretory cells and presynaptic nerve terminals.

The introduction of the patch electrode voltage clamp technique has provided new opportunities to investigate the links between electrical events and endocrine cell function. Single channel recording and control of intracellular composition of single cells are just two of the advantages afforded by this approach. Many of the initial studies have utilized endocrine cells as substrates to further probe the biophysics of ionic channel function. In this symposium, we will attempt to address the broader questions of the interdependence between hormone/secretogogue binding, hormone release, and membrane electrical changes.

The presentations were chosen to represent a variety of vertebrate cell types and to emphasize new approaches to "classical" endocrine questions such as excitation-secretion coupling and feedback regulation. Each speaker will include an assessment of the status of progress towards a particular research goal shared by himself and other collegues. We hope the presentations will serve as a source of information and a stimulus to others to join in similar efforts.

Th-AM-MinI-2 ONIC CHANNELS AND THEIR MODULATION BY SECRETOGOGUES IN CULTURED ANTERIOR PITUITARY CELLS

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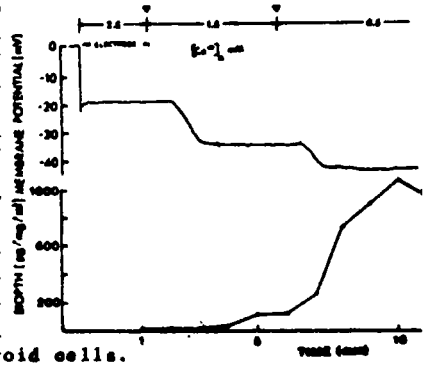
Anterior pituitary cells in tissue culture reveal a variety of responses to hormone releasing- and release-inhibiting factors. Among them are dramatic changes in electrical excitability and intracellular calcium concentrations. Under voltage clamp conditions, neoplastic and normal rat pituitary cells exhibit ionic currents associated with voltage-dependent sodium (I_{Na}), calcium (I_{Ca}) and potassium ($I_K V$) channels and with Ca-activated potassium channels ($I_K Ca$). In cells of the GH tumor line, thyrotropin-releasing hormone (TRH) stimulates prolactin secretion and elevation of intracellular calcium in both a rapid, transient and a prolonged phase. Recordings of membrane potential reveal a transient hyperpolarization followed by increased excitability with TRH application. Under voltage clamp TRH produces a transient (30 sec) increase in $I_K Ca$ followed by a prolonged (4 min.) reduction in $I_K V$. These effects are temporally correlated with the 2 phases of secretion, internal calcium concentration changes, and membrane potential changes. In contrast, no changes are observed in voltage-dependent Ca currents in response to TRH under conditions which experimentally isolate Ca channel currents. Single channel recordings from on-cell patches suggest that the TRH-induced increase in $I_K Ca$ arises from cytosolic [Ca] elevation. Extension of these experiments to other secretogogues and to identified secretory cell types from normal pituitary cultures may provide clues to excitation-secretion coupling mechanisms in these cells.

(Supported by NIH grant NS18788 to G.S. O.).

Th-AM-MinI-3 CALCIUM SENSING MECHANISM IN THE PARATHYROID GLAND: MEMBRANE POTENTIAL CHANGES AND PTH SECRETION **Nels C. Anderson, Jr., Dept. of Physiology, Duke Univ. Medical Center; Bruce Lobaugh, Dept. of Medicine, Duke Univ. Medical Center; George Leight, Dept. of Surgery, Duke Univ. Medical Center; J.T. Posillico, Brigham & Womens Hosp., Harvard Medical School**

<u>In vivo</u> and <u>in vitro</u> experiments have established that the concentration of extracellular ionized calcium is the dominant factor controlling PTH secretion: hypocalcemia stimulates and hyper calcemia inhibits PTH secretion. The goals of these studies are threefold: 1) to characterize the cellular mechanism of calcium sensing in parathyroid cells; 2) to establish the relationship between the secretory stimulus sensing mechanism and PTH secretion; and 3) to apply these results for studies in human parathyroid dysfunction. In 1979 Bruce and Anderson (Am. J. Physiol 236(1):C15-C21, 1979) described a novel electrical response in the mouse parathyroid cell to changes in extracellular calcium concentration. A very steep dependence of the membrane potential on extracellular calcium concentration was observed such that the membrane hyperpolarized approximately 40mV as the calcium concentration was decreased from 2.5mM to 1.5mM. Experiments in which 1 ml/min fractions of superfusate were collected for PTH assay during continuous intracellular microelectrode recording and during changes in extracellular calcium concentration (Figure) have directly established the correlation between electrical and secretory activity in normal human parathyroid cells.



Th-AM-MinI-4 CHANGES IN SURFACE AREA OF MAST CELLS DURING SECRETION DEPEND ON GUANINE NUCLEOTIDES. E. Neher, J.M. Fernandez (Max-Planck-Institut f. biophysk. Chemie, D-3400 Göttingen, F.R.G.) and B.D. Gomperts (University College, GB-London WC1E 6JJ, Great Britain).

Changes in surface area of secreting cells can be monitored by measurements of membrane capacitance[1,2,3]. Rat peritoneal mast cells undergo vigorous morphological changes when stimulated by various secretagogous. These are accompanied by changes in membrane capacitance (surface area) by up to a factor of six[4]. The changes occur stepwise initially with a stepsize distribution agreeing closely with the morphologically determined distribution of granule surface area (assuming a specific capacitance of the granule membrane of 1 $\mu F/cm^2$). This suggests that individual steps in capacitance correspond to the fusion of individual granules with the plasma membrane.

Capacitance changes are being measured in the whole-cell patch-clamp configuration. This implies rapid dialysis of the cell interior with the pipette filling solution. We found that this dialysis disrupts stimulus-secretion coupling for most standard filling solutions. However, addition of GTP-γ-S and of a high energy phosphate (ATP or ITP) invariably led to full secretion in the absence of any additional stimulus. Contrary to expectations, the addition of Ca (extracellularly or intracellularly) was not a requirement for secretion in the presence of 20 μM GTP-γ-S.

[1]L.A. Jaffe, S. Hagiwara, and R.T. Kado, Developmental Biology 67, 243-248 (1978)
[2]J.I. Gillespie, Proc. Roy. Soc. Lond. B 206, 293-306 (1979)
[3]E. Neher and A. Marty, Proc. Natl. Acad. Sci. USA 79, 6712-6716 (1982)
[4]J.M. Fernandez, E. Neher, and B.D. Gomperts, Nature (in press)

Th-AM-MinI-5 MODULATION OF CELLULAR ELECTRICAL PROPERTIES BY PEPTIDE HORMONES. Robert L. DeHaan, Department of Anatomy, Emory University Health Science Center, Atlanta, Georgia 30322.

Occupancy of specific surface receptors by peptide hormones can produce significant electrophysiological responses in a variety of cell types. Well-documented effects include increases in sodium conductance in epithelial apical membranes caused by aldosterone and antidiuretic hormone; modulation of ion fluxes by polypeptide growth factors; and modifications in target cell membrane potential by cholecystekinin and other brain peptides. Insulin is known to cause depolarization of rat hepatocytes and produces a ouabain-insensitive hyperpolarization in skeletal muscle and cardiac tissue. In embryonic heart, nanomolar concentrations of insulin slow the spontaneous beat rate by increasing an outward current. Other peptide hormones such as oxytocin, parathormone and calcitonin also have measurable effects on cell membrane potential. Postulated mechanisms for these effects include: 1) direct activation of receptor/channel complexes by ligand binding; 2) activation of channels through a phosphorylation step or the mediation of second messengers; 3) mobilization and insertion of new channels in the cell membrane. Current investigations in several laboratories will be reviewed, with a focus on our own recent patch-electrode studies of the insulin-activated current in embryonic chick heart cells. (Supported by NIH Grant PO1 HL27385)

Th-AM-MinI-6 IONIC CHANNELS IN PANCREATIC B-CELLS AND THEIR POSSIBLE ROLE IN GLUCOSE INDUCED INSULIN RELEASE. D. R. Matteson. University of Pennsylvania, Philadelphia, PA 19104.

In recent years, microelectrode recordings have shown that pancreatic B-cells generate glucose-dependent periodic electrical activity. During activity, the B-cell membrane potential oscillates between an active phase, consisting of several small spikes superimposed on a plateau-like depolarization, and a hyperpolarized silent phase. This electrical activity might reflect the mechanism by which glucose stimulates insulin secretion from the B-cell, e.g. secretion might be activated by the influx of calcium ions during the active phase.

The development of the patch-clamp technique has made it possible to directly study the ionic channels responsible for electrical activity in B-cells, and to examine the role of these channels in the response of the B-cell to glucose stimulation. The whole-cell variation of the patch-clamp technique has been used to identify and characterize channels by performing voltage clamp experiments on isolated pancreatic islet cells. Inward currents are carried by both sodium and calcium channels and outward currents are carried by at least one type of potassium channel. The relationship between the activity of these ionic channels and glucose induced electrical activity will be discussed.