sible explanation. It has been suggested that myocardial ischemia induces alterations in membrane lipids, lipid fluidity, and altered distribution pattern of intramembranous particles in the mitochondrial membranes (*23*, *24*). Certainly, changes in the hydration and physical properties of water (as measured by proton relaxation times) in mitochondria occur early in the response to ischemic insult. These observations are well correlated with those of some previous studies in which we demonstrated impairment of fatty acid oxidation and accumulation of long-chain acyl ester intermediates early in ischemia.

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8 November 1979; revised 22 February 1980

Electrical Activity in an Exocrine Gland: Optical Recording with a Potentiometric Dye

Abstract. *Propagated action potentials in the salivary gland of a freshwater snail were detected by optical means. Voltage-dependent absorption changes from acinar cells stained with a merocyanine-oxazolone dye faithfully reproduced the time course of electrical activity in this tissue. Such signals may provide a useful tool for the study of endocrine tissue as well.*

A number of exocrine and endocrine gland cells display regenerative voltage changes in response to neural or hormonal stimulation or both (*1*). It is believed that these regenerative electrical events are causally related to the coupling of excitation to secretion (*2*), although the underlying mechanisms remain to be elucidated. Attempts to understand the excitation-secretion process have been impeded in part by the relatively small size of gland cells, which makes intracellular microelectrode recording very difficult (*3*). Electrophysiological study of glands might therefore be facilitated if an alternative technique for monitoring membrane potential could be developed that did not require cell impalement.

Vital dyes that respond optically to rapid changes in membrane potential have been used to monitor electrical activity in a variety of systems including squid giant axons (*4*), invertebrate central neurons (*5*, *6*), and vertebrate cardiac (*7*) and striate (*8*) muscle. To our knowledge, no attempt has been made to utilize these potentiometric probes in any electrophysiological studies of glandular tissue (*9*–*11*). We now report that propagated action potentials in a stained invertebrate salivary gland generate optical "spikes" that are clearly visible in a single oscilloscope sweep (*12*).

To establish the feasibility of an approach to gland electrophysiology based on optical measurement of membrane potential, we focused our efforts on the bilaterally paired salivary glands from the freshwater snail *Helisoma trivolvis*. This exocrine gland was selected since it was recently shown that individual acinar cells generate large overshooting action potentials when electrically or neurally stimulated and that these regenerative responses are propagated throughout the gland because of extensive electrotonic coupling between acinar cells (*13*). Another important technical advantage of this preparation is the relative ease with which intracellular microelectrode recordings can be made from individual acinar cells. This allows changes in the optical signal to be compared directly with membrane potential changes recorded intracellularly.

Surgically isolated snail salivary glands were stained for 20 minutes with a merocyanine-oxazolone dye (NK 2367, Nipon Kankoh-Shikiso Kenkyusho Co., Okayama, Japan) (*6*). To measure extrinsic voltage-dependent changes in absorption, an extended region of the gland was viewed under a water-immersion objective (×20; 0.33 numerical aperture) on a Reichert Zetopan binocular microscope. An adjustable slit in the objective image plane was positioned so that only light that had passed through a selected region (0.05 to 0.1 mm^2) of the gland reached a silicon diode photodetector (PV 444, EG & G, Inc.) (*6*).

Relatively large optical signals could be obtained from glands that had been superfused or luminally perfused with Ringer solution (*14*) containing the dye (Fig. 1). In Fig. 1A, an optical signal from a gland that had been luminally perfused with dye at 200 μg/ml is compared with the signal from another gland that had been superfused with dye at 25 μg/

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ml. In this and the other records, a downward deflection of the optical record represents a decrease in transmission.

The size of the optical signal was dependent on the wavelength of the transilluminating light. The largest signal : noise ratio was obtained with illumination at 676 nm (Fig. 1B). At this wavelength, decreases in membrane potential (spikes) were accompanied by increases in light absorption. Optical signals were also observed at 540 and 720 nm, but at these wavelengths, decreases in light absorption occurred during membrane depolarization. The triphasic nature of the wavelength dependence in snail salivary glands is significantly different from that previously reported for this dye in barnacle central neurons or squid giant axons (*15*), in which membrane depolarization produced decreases in light absorption at all wavelengths tested (*6*). However, a similar triphasic wavelength dependence was observed for a related merocyanine-rhodanine analog (dye XVII) in vertebrate cardiac muscle (*7*).

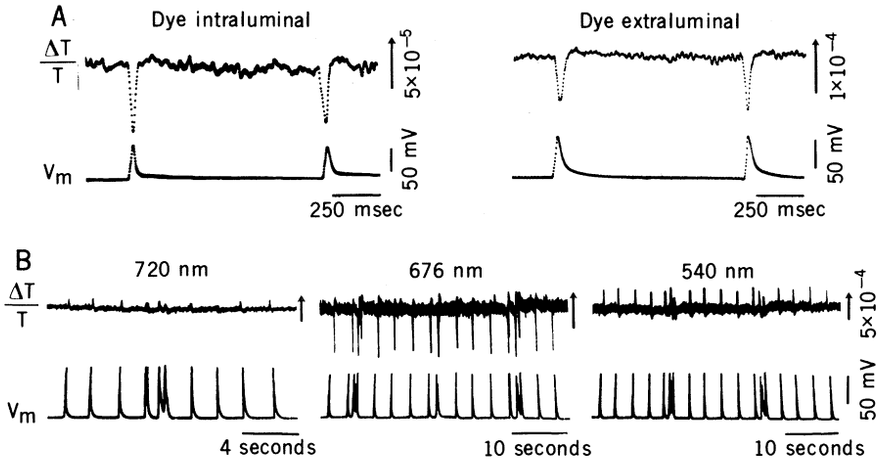

Fig. 1. (A) Optical spikes after intra- and extraluminal staining of the salivary gland. The upper traces represent the light intensity recorded from an extended region of the tissue and the lower traces show the membrane potential (V_m) measured from single acinar cells with a microelectrode. Neural stimulation was used to evoke the first action potential in each record; the second spike resulted from direct electrical stimulation of the gland. In this and the other records, the vertical arrows represent fractional changes in transmitted intensity ($\Delta T/T$), with an increase in light reaching the photodiode shown upward. The optical trace on the left was obtained after staining a gland with a merocyanine-oxazolone dye (200 μg per milliliter of Ringer solution) by luminal perfusion for 15 minutes; the trace on the right was obtained after bathing the outside of the gland in a weaker solution of this dye (25 μg/ml) for 20 minutes. The record on the left is the average of four trials; all the other records were obtained in single trials. Wavelength is 676 ± 26 nm [full width at half-maximum (FWHM)]. High-frequency response-time constants of the light-measuring systems were 260 μsec and 1.9 msec in the left and right records, respectively. The a-c coupling time constant was 425 msec. All records were made at 23°C. (B) Optical signals from salivary gland at three wavelengths. The membrane potential records were obtained with a microelectrode in a single acinar cell throughout the experiment. The dye was applied extraluminally for 15 minutes at a concentration of 25 μg/ml. The interference filters had transmission maxima at 720 ± 15, 676 ± 26, and 540 ± 15 nm (FWHM); records were made in the order 720, 540, and 676 nm. The optical traces were recorded in single long sweeps (20 or 50 seconds) on an oscilloscope screen. In each case, the high-frequency response time constant of the light-measuring system was 1.9 msec. The a-c coupling time constant was 425 msec.

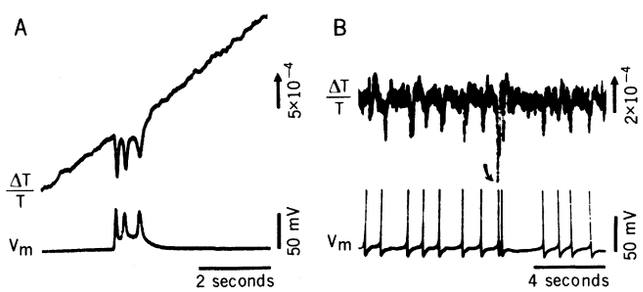

Fig. 2. (A) Optical record of a complex membrane potential wave form. The upper trace shows the change in transmission of the gland at 676 ± 26 nm (FWHM). The optical signal is d-c-coupled with a 12-bit digital sample-and-hold circuit of simple design. The record was obtained without signal averaging, and the sloping baseline results primarily from bleaching of the dye. However, accurate modeling of the slope would require the convolution of this effect with the temperature-dependent shifts in the transmission of the interference filter and the heat filter (KG1, Schott Optical Glass), and the changing overlap with the absorption spectrum of the stained membrane and the silicon photodiode sensitivity curve. The lower trace is the membrane potential recorded from a single acinar cell impaled with a microelectrode. The gland was stained extraluminally with dye (25 μg/ml) for 20 minutes. After some preliminary experiments, the gland was restained for 15 minutes in a stronger solution (50 μg/ml). Response-time constant of the light-measuring system was 1.9 msec. (B) Optical record of spike activity in the salivary gland, illustrating differential invasion of the tissue. The lower record is an intracellular microelectrode recording from the contralateral salivary effector neuron in a preparation in which the paired buccal ganglia were left attached to the salivary gland. The gland was perfused intraluminally with dye (400 μg/ml) for 20 minutes. Wavelength is 676 ± 26 nm (FWHM). Response-time constant of the light-measuring system was 1.9 msec. The a-c coupling time constant was 425 msec.

Figure 2A shows a complex electrical wave form recorded with an intracellular microelectrode from an acinar gland cell. (Although their origins are not understood in terms of ionic currents, such complex wave forms are frequently observed in unstained and apparently normal preparations of this gland.) It can be seen that the complex changes in the membrane potential of a single acinar gland cell are closely mirrored by changes in the optical signal. Although it is not possible to record directly the membrane potential of the innervating nerve fibers, we think it unlikely that similar complex wave forms would be concurrently present. Also, the lack of dispersion in the optical wave form recorded from a large fraction of the gland provides additional evidence for the high degree of electrotonic coupling between adjacent acini in this tissue.

Although we have consistently observed close agreement between microelectrode- and optically recorded gland responses, we have also noted changes in the relative amplitude of the optical signal, even during a single oscilloscope sweep. Since the optical signal is generated by electrical changes over an extended region of the gland, it is possible that differences in the amplitude of the optical signal represent differential invasion of the tissue by the propagated action potential.

In the intact snail, excitatory synaptic input to each side of the salivary gland is provided by a pair of effector neurons in

the left and right buccal ganglia. To study the relation between neural excitation and propagation of the gland action potential, we utilized a preparation in which the paired buccal ganglia were left attached to one side of the salivary gland. Figure 2B shows records from an experiment in which activity in one of the effector neurons was monitored with an intracellular microelectrode while activity in the gland was monitored optically. Firing of the effector neuron evoked "optical spikes" in the gland reflecting the propagation of gland action potentials that followed the neuronal activity at a constant latency. The arrow indicates a large optical spike that preceded firing in the effector neuron and that probably arose from another excitatory input. Since it was not possible to monitor activity in both effector neurons simultaneously, we cannot be certain that the larger optical spike was the result of activity in the other effector neuron. However, it is clear that different sources of excitatory inputs to the gland can evoke optical spikes of significantly differing amplitudes. Furthermore, it is reasonable to assume that the differences in the optical signal reflect differences in the degree to which a regenerative spike propagates through the gland. A photodiode array, monitoring simultaneously the optical signals from a large number of discrete regions of the gland, could be used to study conduction pathways in this and other electrical syncytia.

These results demonstrate the feasibility of optically monitoring membrane potential in an exocrine gland. We believe that it will be possible to use this approach in electrophysiological studies of other exocrine tissues. Further, we hope that this technique may prove a powerful tool for the study of excitation-secretion coupling in endocrine glands.

Note added in proof: Ross and Reichardt (*17*) found a similar triphasic wavelength dependence of the optical signals from rat superior cervical ganglion cells with the merocyanine-oxazolone and merocyanine-rhodanine dyes.

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5 December 1979

Bacterial Origin of Luminescence in Marine Animals

Abstract. *Bacterial luciferase activity was detected in light organ extracts of squids, fishes, and pyrosomes, suggesting that these systems are derived from bacteria-animal symbioses. In none of these cases was it possible to culture luminous bacteria. Analyses of the decay kinetics show that the luciferases from the squid, ceratioid, and pyrosome light organs are all similar to bacterial luciferases from the genus* Photobacterium, *while those from the anomalopid light organs are different.*

Bioluminescence is common in marine organisms; estimates are that 90 percent or more of midwater animals are capable of light emission (*1*, *2*). In bacteria and protists, the entire organism is usually luminous, but as structural complexity increases there is a tendency for light emission to be restricted to specialized organs. Such bioluminescence in marine fishes and squids may be an intrinsic property of the animal tissue in photophores, or may emanate from symbiotic bacteria harbored in specialized light organs (*3*). The symbioses exhibit species specificity; members of a given fish or squid family always have the same species of bacteria associated with them (*4*). Of the two recognized genera of marine luminous bacteria, *Beneckea* and *Photobacterium*, only species of *Photobacterium* have been found as light organ symbionts. However, in some cases the distinction between symbiotic and intrinsic light emission is not easily made; bacteria-like bodies (bacteroids) are present in some light organs, but no luminous bacteria have been cultured from them (*2*, *3*, *5*-*8*). We describe here enzyme methods that can be used in such cases to establish the presence and identity of bacterial symbionts.

Bacterial luciferase, a mixed-function oxidase, catalyzes light emission via the reaction shown in Fig. 1. The enzyme is found only in prokaryotes, and thus is not found in nonbacterial luminescent systems. An unusual feature of the enzyme is its slow rate of turnover, which is dependent on the aldehyde used in the in vitro reaction (*9*). If dodecanal is the aldehyde used, luciferases isolated from the two bacterial genera are different in their kinetic properties; those from *Beneckea* exhibit slow decay kinetics, while those from *Photobacterium* give fast decay (Fig. 1, inset). On the basis of this difference, luciferase decay kinetics have been used to identify the bacterial genus from which the luciferase was extracted (*10*).

In our study, light organ extracts of several fishes, squids, and tunicates were examined (Table 1). Bacterial luciferases were detected and kinetically characterized, even though the bacterial symbionts could not be cultured (*11*).

Extracts from the light organs of six specimens of the squid, *Heteroteuthis hawaiiensis*, were tested. All were positive for bacterial luciferase and showed fast decay kinetics, characteristic of the genus *Photobacterium*.

Extracts of two specimens of *Pyrosoma* sp. (Tunicata, Pyrosomida) were

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