

Large and Rapid Changes in Light Scattering Accompany Secretion by Nerve Terminals in the Mammalian Neurohypophysis

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ABSTRACT Large changes in the opacity of the unstained mouse neurohypophysis follow membrane potential changes known to trigger the release of peptide hormones. These intrinsic optical signals, arising in neurosecretory terminals, reflect variations in light scattering and depend upon both the frequency of stimulation and $[Ca^{2+}]_i$. Their magnitude is decreased in the presence of Ca^{2+} antagonists and by the replacement of H_2O in the medium by D_2O . These observations suggest a correspondence between the intrinsic optical changes and secretory activity in these nerve terminals.

INTRODUCTION

The release of neurotransmitter substances from vertebrate nerve terminals occurs within milliseconds of membrane depolarization (Katz, 1969) and is detected experimentally through the electrical consequences of transmitter binding to receptors in the postsynaptic membrane. The study of excitation-secretion coupling at vertebrate nerve terminals and elsewhere would be powerfully facilitated if it were possible to monitor directly both the intracellular potential change and the secretory event itself, with appropriate temporal resolution. Conventional electrophysiology and biochemical assays (Petersen, 1980; Poulain and Wakerley, 1982; Douglas, 1978; Nordmann, 1983) offer complementary insights into neurosecretory behavior, but rapid detection of the secretory event itself is absent.

The vertebrate hypothalamo-neurohypophysial system represents a classical model for the study of excitation-secretion coupling at nerve terminals (Douglas, 1963, 1978; Douglas and Poisner, 1964; Nordmann, 1983; Poulain and Wakerley, 1982). Magnocellular neurons located in the hypothalamus (supraoptic and paraventricular nuclei in mammals; preoptic nucleus in lower vertebrates) project their axons as bundles of fibers through the median eminence and

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infundibular stalk to terminate in the neurohypophysis, where the neurohypophysial peptides and proteins are secreted into the circulation. Biochemical assays of the calcium-dependent release of peptide hormones have been conducted under a variety of experimental conditions (Douglas, 1963, 1978; Douglas and Poisner, 1964; Nordmann, 1983; Poulain and Wakerley, 1982). Although the nerve terminals of the neurohypophysis are very small (0.5–1.0 μm), we have shown recently (Salzberg et al., 1983; Obaid et al., 1983) that it is possible, using extrinsic optical methods based upon linear potentiometric probes, to record action potentials from synchronously activated populations of terminals in the intact amphibian and mammalian neurohypophysis. In the frog, these recordings provided evidence for sodium and calcium components, and revealed the presence of an afterhyperpolarization that probably reflects a calcium-mediated potassium conductance (Salzberg et al., 1983; Obaid et al., 1985).

We demonstrate here that large intrinsic optical signals accompany and immediately follow the action potential in the terminals of the unstained neurohypophysis of the CD-1 mouse. These signals, recorded without averaging as transmitted intensity (transparency) changes in the image plane of a compound microscope, reflect variations in large-angle light scattering rather than absorbance. A component of the optical signal appears to mark the arrival of the impulse in the terminals, while a larger component seems to monitor an early event in the neurosecretory process. The fractional changes in transparency, in the latter case, depend upon the frequency of stimulation and $[\text{Ca}^{2+}]_o$ and are diminished in the presence of Ca^{2+} antagonists and D_2O . Some of these results have appeared in preliminary form (Salzberg et al., 1984; Obaid et al., 1984).

METHODS AND RESULTS

Optical Detection of Secretory Events in Nerve Terminals

Fig. 1A shows an optical recording (Cohen and Salzberg, 1978; Salzberg, 1983; Grinvald, 1985) of a train of action potentials in a population of nerve terminals in the neurohypophysis of the CD-1 mouse (Charles River Breeding Laboratories, Wilmington, MA). The preparation had previously been incubated in a Ringer's solution (in mM: 154 NaCl, 5.6 KCl, 1 MgCl_2 , 2.2 CaCl_2 , 10 glucose, 20 HEPES, adjusted to pH 7.4 with NaOH) containing 0.1 mg/ml of the potential-sensitive merocyanine-oxazolone dye NK 2367 (Salzberg et al., 1977). The method that we have used for multiple-site optical recording is described fully elsewhere (Salzberg et al., 1977, 1983; Grinvald et al., 1981). Here the neurointermediate lobe of the mouse pituitary was imaged onto a photodiode matrix array using a 10 \times , 0.4 numerical aperture (NA) objective (Wild Heerbrugg Instruments, Inc., Farmingdale, NY) modified for pseudo-water immersion by sealing the front element, and the extrinsic absorption changes that monitor action potentials in the terminals (Salzberg et al., 1983; Obaid et al., 1983) were confined to elements of the photodetector array that correspond to the neurohypophysis. The record in Fig. 1A represents the change in transmitted light intensity at 675 nm, monitored in a single sweep by one channel of a 124-channel, computer-based system for multiple-site optical recording of transmembrane voltage (MSORTV) (Salzberg et al., 1977, 1983; Grinvald et al., 1981, 1982; Senseman et al., 1983),

during seven stimulations of the axons of the infundibular stalk at 16 Hz. The fibers of the infundibular stalk branch extensively and their terminals constitute most of the excitable membrane in the pars nervosa (Dellman, 1973; Nordmann, 1977; Castel et al., 1984). The trace in Fig. 1A exhibits a gradual decline in the baseline, followed by recovery, having the appearance of a transient hyperpolarization. The constant size of the action potential upstroke, however, suggests that this apparent hyperpolarization may not represent a change in membrane potential. Fig. 1B shows a single trial recorded by the same detector element, except that the measuring wavelength was 540 nm. At this wavelength, the potential-dependent absorption change exhibited by NK 2367 is known to be opposite in sign to that observed at 675 nm (Senseman and Salzberg, 1980). Notice, however, that although the optical spikes are reversed in sign, the slow component of the signal is essentially unchanged. This weak dependence on wavelength of the slow component of the optical trace suggested that it might reflect a change in light scattering.

Fig. 1C illustrates an identical experiment, carried out on another mouse neurohypophysis, before staining. Here the dye-dependent extrinsic absorption signal that depends upon membrane potential is absent, revealing a series of increases in transmitted light intensity (downward deflections of the trace), each preceded by a transient decrease in transparency (upward deflection) occurring at the time of the action potential. This experiment employed a relatively long AC coupling time constant (3 s) in order to minimize the distortion of the time course of the optical signal. Nonetheless, the tendency toward recovery following the train of stimuli is largely an artifact of the coupling time constant. A similar experiment is illustrated in Fig. 1D, in which a digital sample-and-hold circuit, consisting of 12-bit analog-to-digital and digital-to-analog converters connected in series (Senseman and Salzberg, 1980), is used instead of capacity coupling. This method avoids any AC distortion of the signal, which is observed to remain nearly constant for seconds after the cessation of the stimuli.

Fig. 1E illustrates the wavelength dependence of these intrinsic optical signals between 600 and 860 nm, corrected for the spectral efficiency of the apparatus. The wavelength range was selected to exclude major absorption peaks of intrinsic pigments, e.g., hemoglobin. The fractional change in transmitted intensity exhibits a gradual decline with increasing wavelength, which is consistent with a change in light scattering. All components of the optical signals were eliminated when the neurohypophysis was depolarized with KCl (not shown).

Optical techniques that employ visible light, especially those that rely upon intrinsic optical changes that require no exogenous probes, offer unique capabilities in terms of resolution in time and space, combined with a gentleness that permits the nondestructive measurement of many cellular and subcellular processes. In particular, light-scattering methods have been applied to biological systems, *in vitro* and *in vivo*, since Tyndall (1876) (see Earnshaw and Steer, 1983), and have played a role in a variety of recent efforts to study changes in neuron structure during action potential propagation and synaptic transmission (Cohen, 1973). Cohen and Landowne (1970) reported small (8×10^{-7}) changes in light scattering in the skate electric organ that appeared to be presynaptic in origin, and Shaw and Newby (1972) detected calcium-dependent movement in

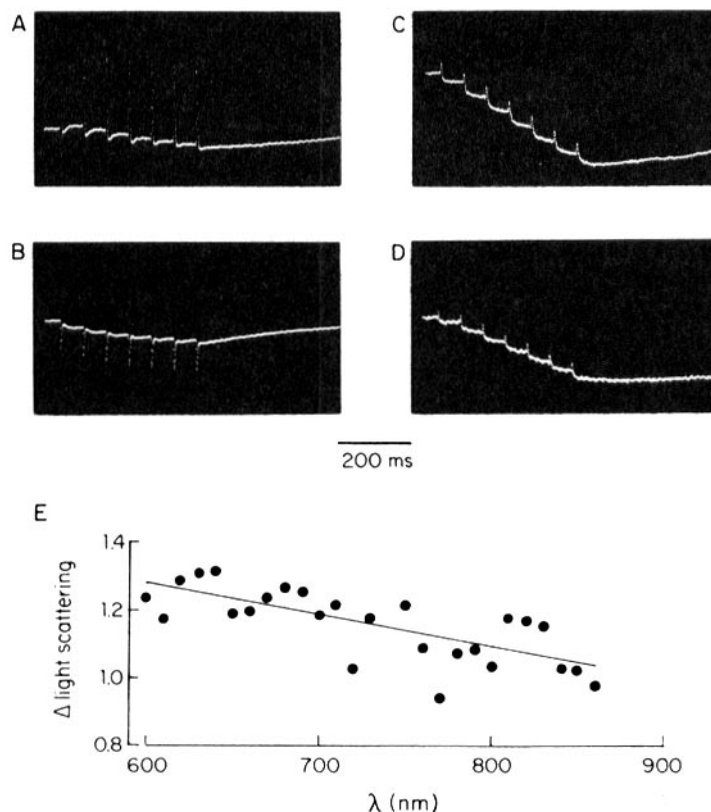


FIGURE 1. Extrinsic and intrinsic optical changes recorded from nerve terminals in the neurohypophysis of the mouse. (A) Changes in the extrinsic absorption at 675 nm (52 nm full width at half-maximum) of nerve terminals after staining for 25 min in 0.1 mg/ml NK 2367. A single train of 500- μ s (60 V) stimuli at 16 Hz was delivered to the posterior pituitary through the axons of the infundibulum for 400 ms. The resulting changes in transmitted light intensity are shown, recorded by a single element of a 144-element photodiode matrix array, with an upward deflection of the trace, here, and in succeeding figures, representing a decrease in intensity. The AC coupling time constant for the light measurement was 400 ms. The fractional changes in light intensity during the action potentials were \sim 0.3%. Single sweep. MPP013. (B) Same experiment as in A, except that the transmitted intensity was monitored at 540 nm (60 nm full width at half-maximum). At this wavelength, the voltage-dependent extrinsic absorption change exhibited by the merocyanine-oxazolone probe NK 2367 is known to be opposite in sign to that observed at 675 nm. MPP013. (C) Intrinsic optical changes recorded at 675 nm (52 nm full width at half-maximum) from the nerve terminals of an unstained neurohypophysis. The preparation was stimulated as in A, and the resulting changes in transmitted light intensity recorded by a single element of the photodetector array are shown. Here, again, an upward deflection of the optical trace represents a decrease in transmitted light intensity or an increase in opacity. This is probably equivalent (see panel E) to an increase in large-angle light scattering. The AC coupling time constant for the light measurement was 3 s. The intrinsic optical signal, resulting from the train of stimuli shown here, corresponds to a fractional change in intensity of \sim 0.2%. Single

a thoracic ganglion of a locust (*Schistocerca gregaria*) by monitoring the power spectrum of "twinkling" (light beating) produced by scattered laser light (see also Piddington and Sattelle, 1975; Englert and Edwards, 1977). These preliminary reports, however, have not been pursued.

In the absence of potentiometric dye staining of the terminals of the neurohypophysis, two intrinsic optical signals, illustrated in Fig. 1 *C*, are evident after each stimulus. The rapid upward deflection of the trace (decrease in transparency), which we term the "E-wave," appears to coincide with the arrival of the action potential at the terminals (cf. Fig. 1 *A*), while the subsequent increase in transparency, the "S-wave," appears to correspond to a slower and longer-lasting process occurring in the nerve terminals. Several lines of evidence suggest that this latter intrinsic optical signal (Fig. 1, *C* and *D*) is closely correlated with the secretory process. If this association is more than accidental, we would anticipate that the later changes in transparency (but not the E-wave) would exhibit certain properties that characterize neurosecretory systems in general and the release of neuropeptides in particular, viz., dependence on stimulation frequency, with marked facilitation, dependence on extracellular Ca^{2+} concentration, and sensitivity to Ca^{2+} antagonists and to various pharmacological procedures known to influence secretion (see below).

The frequency dependence of the transparency changes (intrinsic optical signals), which we refer to henceforth as "light scattering" (see Discussion), is illustrated in Fig. 2. In each panel, the stimuli are delivered to the neurohypophysis for 400 ms, but at different frequencies. Panels *A–D* show a monotonic increase in the total size of the light-scattering change with number of stimuli. The size of the fractional change in light scattering per stimulus ($\Delta\text{light scattering}_{(50-460 \text{ ms})}/N$) varies with frequency, reaching a broad maximum at between 8 and 16 Hz. Fig. 2*D* demonstrates the tendency of the total optical change to saturate at very high stimulation rates. In each instance, a marked facilitation of the optical response is evident between the first and second stimulus. Panel *E* was obtained under conditions identical (16 Hz) to those of panel *C*, except that 1 mM CdCl_2 was added to the bath for 15 min before the single trial recorded here. Cadmium ions are known to block calcium currents in a variety of preparations (see, for example, Standen, 1981) and they have recently been shown to block a calcium component of the action potential in nerve terminals in the neurohypophysis of *Xenopus* (Salzberg et al., 1983; Obaid et al., 1985). In Fig. 2*E*, Cd^{2+} is seen to block >90% of the S-wave (downward

sweep. MPP042. (*D*) Experiment similar to *C*, except that the optical signal was DC-coupled using a digital sample-and-hold circuit that eliminated the distortion introduced by capacitance coupling. Unstained preparation, 675 nm (52 nm full width at half-maximum). Single sweep. MPP067. (*E*) Wavelength dependence between 600 and 860 nm of the intrinsic optical changes recorded from nerve terminals of the unstained mouse neurohypophysis. The fractional intensity changes have been corrected for the efficiencies of the apparatus and for the gradual deterioration of the preparation. The stimulus trains were delivered at 3-min intervals. All trials were carried out in normal Ringer's solution. The rise time of the light-measuring system (10–90%) was 1.1 ms. MPP061.

deflection of the trace; decrease in opacity) of the scattering change without significantly affecting the rapid upstroke (E-wave) that occurs coincidentally with the action potential. The plot in Fig. 3 summarizes the dependence of the magnitude of the light-scattering change on the rate and number of stimuli and also includes the effect of barium ions (see below).

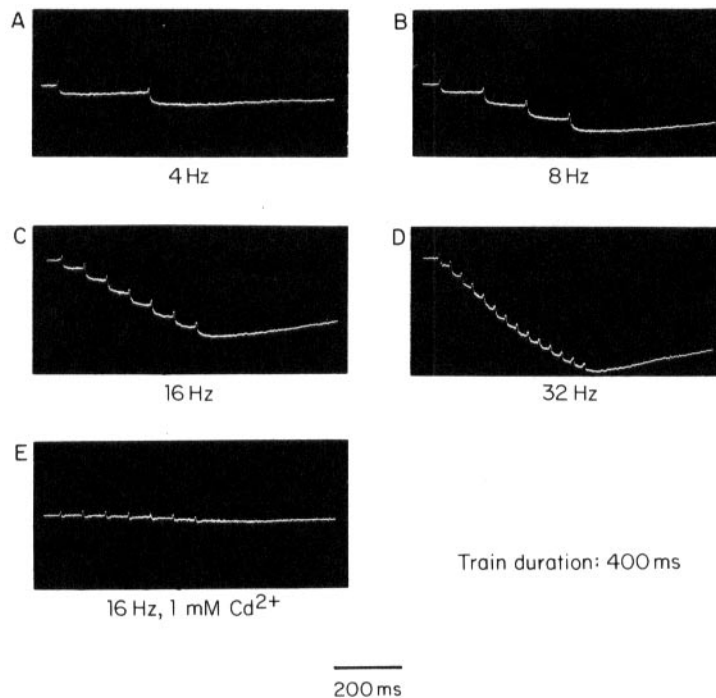


FIGURE 2. Frequency dependence and Cd^{2+} block of the light-scattering change in nerve terminals. (A–D) Intrinsic optical signals at 675 nm resulting from stimulation of the terminals of the mouse pars nervosa at 4, 8, 16, and 32 Hz, respectively, for 400 ms, in normal Ringer's solution. (E) Experiment identical to that in C, except that the intrinsic optical signal was recorded 15 min after the addition of 1 mM Cd^{2+} to the Ringer's solution bathing the neurohypophysis. AC coupling time constant, 3 s; rise time of the light-measuring system (10–90%), 1.1 ms. Temperature, 23°C. MPP036.

Effects of Extracellular Calcium

Calcium ion profoundly influences neurosecretory activity (e.g., Katz, 1969; Douglas, 1978; Dodge and Rahamimoff, 1967) and the extracellular concentration of this ion would be expected to modulate the size of an intrinsic optical signal related to secretion from the terminals of the neurohypophysis. Fig. 4 shows the effect of $[\text{Ca}^{2+}]_o$ on the light-scattering signal evoked by stimulation of the infundibulum at 10 Hz for 400 ms. Traces A–F, which are each the average of 16 sweeps, were obtained in the alphabetical order shown. The effect of calcium concentration is apparent, and the blockade of the optical signal by 0.2

mM Cd^{2+} in Fig. 4F is dramatic. Calcium ion, of course, has other effects, including a direct effect on excitability (e.g., Frankenhaeuser and Hodgkin, 1957), and these must be considered. To eliminate the effects of threshold variation, the stimuli were supramaximal in each instance. Examination of the records in Fig. 4 (compare, for example, B and D) reveals significant differences in the amplitudes of the E-waves that precede each S-wave in the light-scattering signal and coincide in time with the terminal action potential. The E-wave evidently has an origin different from that of the large S-wave that is blocked by

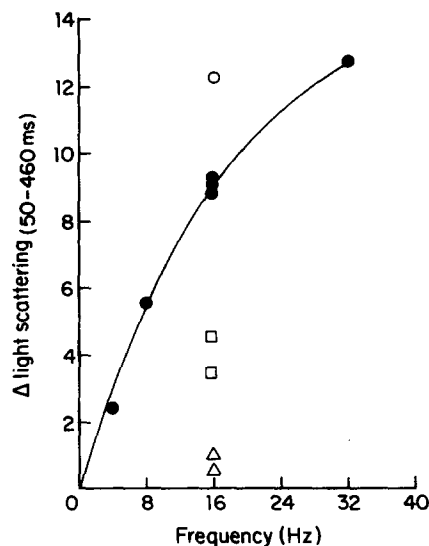


FIGURE 3. Fractional change in transparency between 50 and 460 ms, as a function of stimulation frequency, after stimulation for 400 ms. The data are not corrected for the absolute number of stimuli. At 16 Hz, data are also plotted for three trials from the same experiment, in which 1 mM Cd^{2+} was added to the bath (Δ), 2.2 mM Ba^{2+} was substituted for the Ca^{2+} in the bath (\square), and 2.2 mM Ba^{2+} was added to the normal Ringer's solution (\circ); \bullet , control. A-E were single sweeps. Parameters are as in Fig. 2. MPP036.

cadmium. We suggest that this very early intrinsic optical change (E-wave) reflects the arrival of the action potential at the terminals of the neurohypophysis, and we have assumed that, as a compound optical signal, its size is roughly proportional to the number of terminals activated at a given time. On this assumption, we have compensated for changes in the invasibility of the tissue resulting from changes in extracellular calcium by normalizing the S-wave according to the size of the initial E-wave. Two kinds of difficulties attend this interpretation. First, the peak of the E-wave occurs slightly before the peak of the voltage change in the terminals, as monitored by the voltage-sensitive dyes. Although the extrinsic absorption signals could be distorted if the dye response exhibited a slow component, the merocyanine-rhodanine and merocyanine-oxazolone dyes, at the concentrations used in these experiments, respond in $<2 \mu\text{s}$ to step changes in

membrane voltage (Bezanilla, F., A. L. Obaid, and B. M. Salzberg, unpublished observations). If the E-wave depended on inward current, it might be expected to reach a maximum at the time of the maximum rate of rise of the extrinsic absorption (voltage) signal. In fact, the time to peak of this early component of

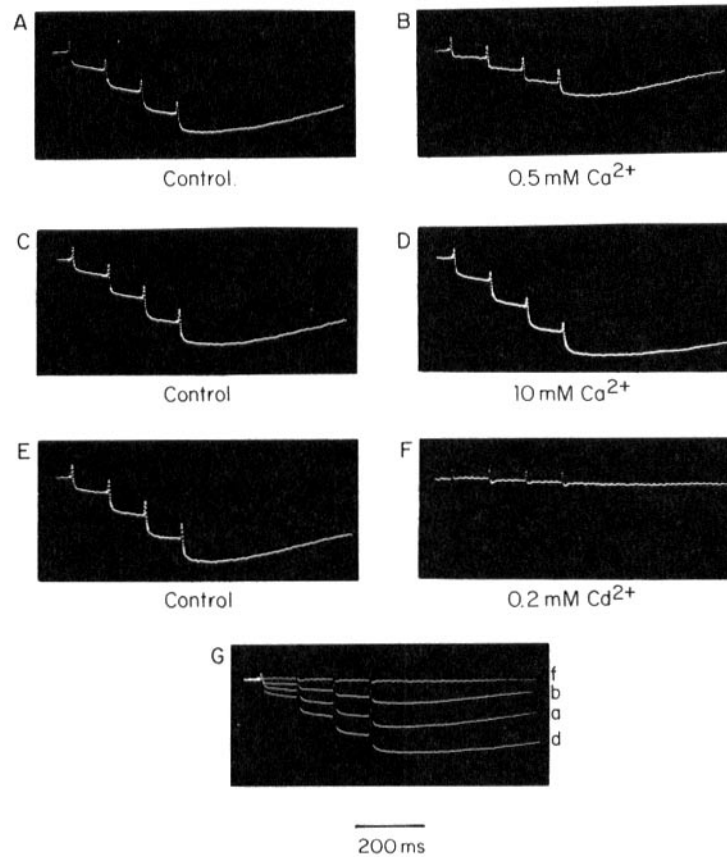


FIGURE 4. Effects of extracellular calcium on the light-scattering changes recorded from the nerve terminals of the neurohypophysis. (*A*, *C*, and *E*) Light-scattering changes accompanying stimulation at 10 Hz of an unstained mouse neurohypophysis in normal Ringer's solution (2.2 mM Ca²⁺). (*B*) Light-scattering changes in the same preparation 20 min after the reduction of the extracellular calcium concentration to 0.5 mM by Mg²⁺ substitution. (*D*) Light-scattering changes in the same preparation 40 min after exposure to Ringer's solution containing 10 mM Ca²⁺. (*F*) Light-scattering changes in the same preparation 10 min after the addition of 0.2 mM Cd²⁺ to the bathing solution. Records *A*–*F* are each the average of 16 sweeps and were recorded in the order shown. (*G*) Traces *a* (control), *b* (0.5 mM Ca²⁺), *d* (10 mM Ca²⁺), and *f* (0.2 mM Cd²⁺) are shown superimposed, after normalization to the height of the first E-wave (see text). AC coupling time constant, 3 s; rise time of the light-measuring system (10–90%), 1.1 ms. Temperature, 24°C. MPP054.

the opacity change lies between the time to peak of the voltage change and the time to peak of the time derivative of the voltage change. This result suggests that the interpretation of the E-wave will be complicated, and although this portion of the signal is related to excitation, it may exhibit both current and potential dependence (Cohen et al., 1972*a, b*).

A second potential difficulty involves our assumption that the size of the E-wave, whether related to voltage or current, is proportional to the number of active terminals. This cannot be better than approximately correct, since it will be affected by changes in the magnitude of the upstroke of the terminal action potential, particularly those resulting from changes in the resting membrane potential. It will also be sensitive to changes in the temporal dispersion of excitation of the terminals, and small changes in the size of the factor used for normalizing the optical signals might seriously distort the quantitative analysis of the optical signals. On balance, however, under constant stimulation conditions, the height of the E-wave would seem to provide a rough comparative estimate of the degree of invasion of the tissue and thereby offer a convenient normalization for the larger light-scattering changes that appear to be related to secretion (S-wave). Moreover, none of the qualitative conclusions that follow are affected by this procedure. Accordingly, Fig. 4*G* shows records obtained in normal Ringer's solution (*a*), together with records obtained in low $[Ca^{2+}]_o$ (*b*), high $[Ca^{2+}]_o$ (*d*), and normal Ringer's solution to which 0.2 mM Cd^{2+} was added (*f*), all normalized so as to equalize the sizes of the E-waves. In this way, the effect of $[Ca^{2+}]_o$ on the light-scattering signal, per active terminal, is demonstrated most clearly.

Perhaps a more dramatic illustration of the profound effect of extracellular calcium concentration on the light-scattering signal elicited by electrical stimulation is shown in Fig. 5. Here, instead of signal-averaged records, single sweeps are presented (Fig. 5, *A-C*) and $[Ca^{2+}]_o$ is reduced (Fig. 5*B*) to 100 μM (with Mg^{2+} replacement). In the low- Ca^{2+} record, the S-wave is entirely eliminated.

Deuterium Oxide Decreases the Magnitude of the Light-scattering Response to Stimulation

Heavy water (deuterium oxide) depresses excitation-contraction coupling in different muscle types (Kaminer, 1960; Svensmark, 1961; Bezanilla and Horowicz, 1975) and excitation-secretion coupling in a variety of systems including pancreatic beta cells (Lacy et al., 1972). In the pars nervosa of the mouse, complete (>98%) replacement of the water in the Ringer's solution by D_2O resulted in a reduction in the size of the light-scattering signal by ~60%. Fig. 6 illustrates the effect of D_2O substitution on the optical response to stimulation of the terminals of the mouse neurohypophysis. Panels *A-C* show light-scattering changes at 675 nm obtained during single trials in normal Ringer's solution (*A*), 30 min in D_2O -substituted Ringer's solution (*B*), and 40 min after a return to normal Ringer's solution (*C*). The depression produced by deuterium oxide seems to reflect primarily a decrease in the light-scattering signal from each active terminal, which is consistent with earlier observations of the effects of D_2O (Kaminer, 1960; Svensmark, 1961; Bezanilla and Horowicz, 1975; Lacy et

al., 1972), rather than a decrease in the number of active terminals, as judged by the small variation in the magnitude of the E-wave. This is demonstrated in Fig. 6D, where the averages of 16 trials are shown, each record obtained immediately after the corresponding single trial. These records have been normalized to the height of the first peak to compensate for small differences in

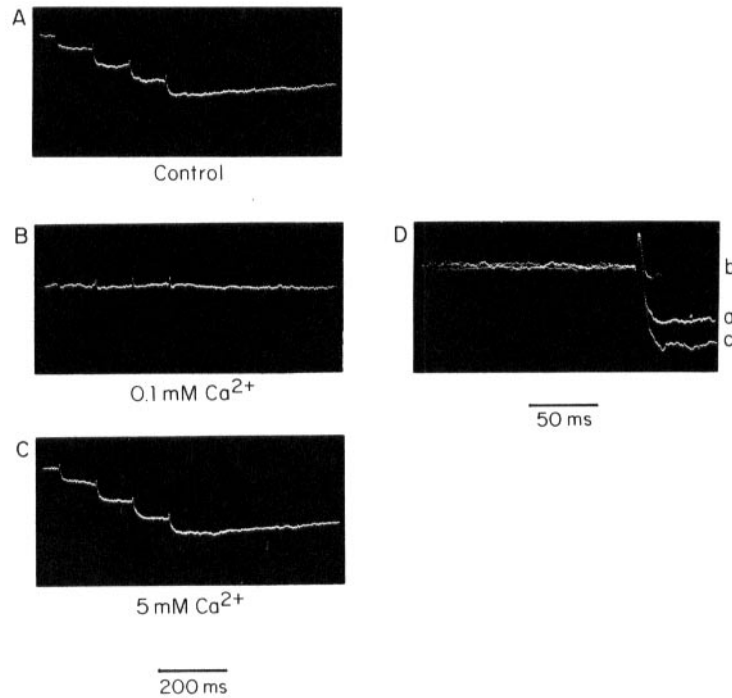


FIGURE 5. Reduced extracellular calcium abolishes the S-wave of the light-scattering signal. (A) Light-scattering changes accompanying stimulation at 10 Hz of an unstained mouse neurohypophysis in normal Ringer's solution (2.2 mM Ca^{2+}). (B) Light-scattering changes in the same preparation 50 min after a reduction of the extracellular calcium to 0.1 mM by Mg^{2+} substitution. (C) Light-scattering changes accompanying stimulation at 10 Hz in the same preparation 30 min after an increase in $[\text{Ca}^{2+}]_o$ to 5 mM. Records A, B, and C were each recorded in a single sweep. (D) Averages of 16 trials recorded under the same conditions as traces A, B, and C. These records were obtained immediately after the corresponding single trains, and are shown here normalized to the height of the E-wave (see text). AC coupling time constant, 3 s; rise time of the light-measuring system (10–90%), 1.1 ms. Temperature, 24–26°C. MPP057.

the number of active terminals. It should be noted that replacement of water with deuterium oxide produces a shift in the true pD, compared with the value measured with a glass pH electrode (Glascoe and Long, 1960), of ~0.4 pD units in the "alkaline" direction. To control for the possibility that the depression of the light-scattering change shown in Fig. 6 resulted from a pD shift, we repeated these experiments in normal Ringer's solution with its pH adjusted to 8.0. This had no effect on the normal light-scattering signals.

Effect of Increasing the Extracellular Volume Fraction with Hypertonic Medium

Rendering the Ringer's solution bathing the neurohypophysis hypertonic with sucrose is expected to increase the volume of the extracellular space by shrinking the terminals transiently. We might detect this effect qualitatively in several different ways, but perhaps the simplest would be to note the effect of hypertonic

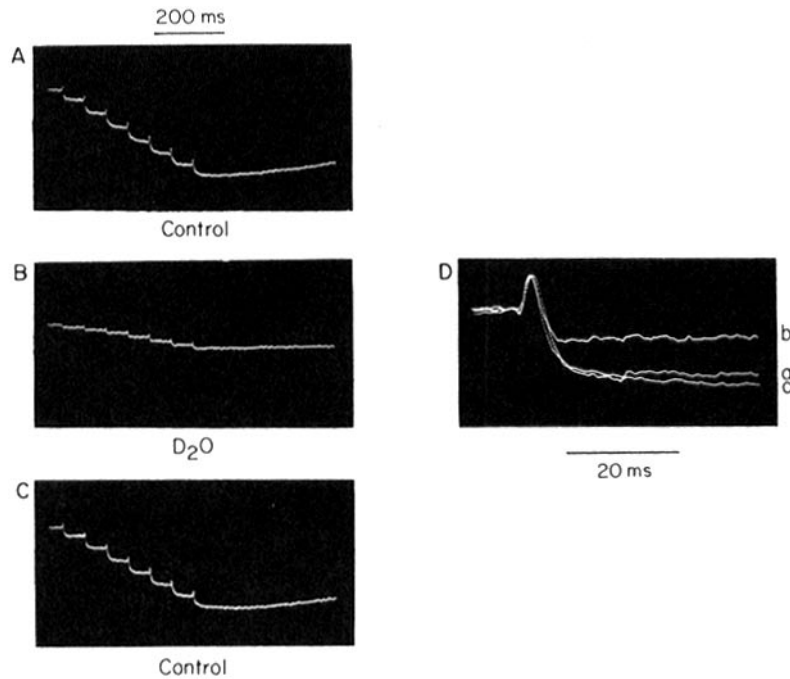


FIGURE 6. Depression of the light-scattering changes by D_2O . (A) Light-scattering changes at 675 nm after a single train of stimuli delivered to the neurohypophysis at 16 Hz for 400 ms, in control Ringer's solution. Single sweep. (B) Light-scattering changes at 675 nm in the same preparation 31 min after the substitution of >98% of the H_2O in the Ringer's solution by D_2O . Single sweep. (C) Same as A, 41 min after a return to normal Ringer's solution. Single sweep. (D) Light-scattering responses to single stimuli, obtained immediately after the corresponding trains. The records are shown superimposed after normalization to the height of the E-wave. Averages of 16 sweeps, stimulated every 5 s. AC coupling time constant, 3 s; rise time of the light-measuring system (10–90%), 1.1 ms. Temperature, 23°C. MPP071.

nicity on the effect of extracellular potassium accumulation (Frankenhaeuser and Hodgkin, 1956). Fig. 7 illustrates this effect indirectly, in a neurohypophysis that had been stained with the merocyanine-oxazolone dye NK 2367 (Salzberg et al., 1977). Fig. 7A shows the sum of the extrinsic absorption change, representing membrane potential, and the light-scattering signal during stimulation at 16 Hz for 400 ms in normal Ringer's solution. The decrease in the overall size of the action potential (20% between the first and last spike in the train) suggests that in this preparation, sufficient potassium is accumulating extracellularly to affect

the undershoots of the action potentials, but that this effect is masked by the downward deflection produced by the S-wave of the light-scattering signal. When the solution bathing the preparation is made 20% hypertonic by the addition of sucrose, the reduction in spike height is less pronounced (~8%), as though the effective extracellular volume were increased by the shrinking of the terminals. The light-scattering signal is, however, not apparently reduced.

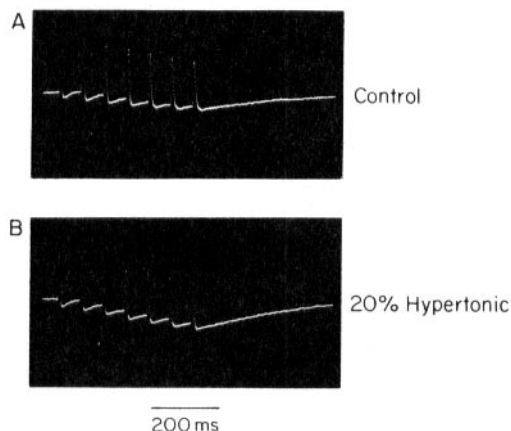


FIGURE 7. Effects on intrinsic and extrinsic optical signals of increasing the extracellular volume fraction with hypertonic saline. (A) Extrinsic and intrinsic optical signals at 675 nm recorded in a single sweep during stimulation at 16 Hz of a mouse neurohypophysis that had been stained with 25 $\mu\text{g}/\text{ml}$ NK 2367 for 25 min. Control Ringer's solution. The progressive decline in the height of the action potentials probably reflects the accumulation of K^+ in the extracellular space. The downward light-scattering signal masks the Frankenhaeuser-Hodgkin effect on the envelope of the undershoots. (B) Same experiment as in A, after 10 min exposure to a Ringer's solution made 20% hypertonic by the addition of sucrose. Extracellular accumulation of K^+ is less evident here (nearly constant peak heights and reduced Frankenhaeuser-Hodgkin effect), while the light-scattering component of the signal is not decreased. Single sweep. AC coupling time constant, 400 ms; rise time of the light-measuring system (10–90%), 1.1 ms. Temperature, 19°C. MPP031.

DISCUSSION

Connection of the Large Intrinsic Optical Signal to Secretory Events at the Nerve Terminals

The intrinsic optical signals reported here are closely correlated with the secretory activity of the magnocellular neuron terminals located in the neurohypophysis of the mouse. A mechanical artifact associated with the contraction of vascular smooth muscle cannot be ruled out unequivocally, and indeed such an effect would be expected to exhibit many of the same properties as an intrinsic signal related to secretion. However, the time course of the S-wave, the component of the light-scattering change that we associate with secretion, would seem

to be too fast by at least an order of magnitude to arise from smooth muscle contraction. The S-wave reaches its half-maximal value within 7–8 ms of the application of the stimulus pulse to the region of the pars nervosa where the infundibular stalk enters (and 3–4 ms after the peak of the extrinsic signal.) Even neglecting a conduction latency, this is faster than any known mechanical response of vascular smooth muscle. Similarly, changes related to the terminal action potential alone do not seem likely to account for the major component of the optical signal. For example, extracellular accumulation of potassium ion might give rise to a change in light scattering, mediated by a transport number effect (Girardier et al., 1963; Barry and Hope, 1969; Cohen et al., 1972*b*) resulting from an increased salt concentration immediately outside the terminals. At least two kinds of evidence make this unlikely. First, the replacement of all of the calcium in the bath by barium ion reduces the light-scattering signal by only ~50% in 10 min (Fig. 3); this occurs despite the fact that barium should not replace calcium in the mediation of a potassium conductance (g_{KCa}) (Standen and Stanfield, 1978; Vergara and Latorre, 1983), and barium is likely, in fact, to reduce the voltage-dependent potassium conductance (Armstrong and Taylor, 1980; Eaton and Brodwick, 1980; Armstrong et al., 1982). Thus, the accumulation of potassium is expected to be severely decreased under these conditions. On the other hand, extracellular barium does replace calcium in some secretory systems (Dicker, 1966; Rubin, 1974), although it is not as effective. Perhaps more significant is the observation that the addition of 2.2 mM barium to the normal complement of extracellular calcium increases the light-scattering signal (Fig. 3), although, again, potassium accumulation might be expected to be reduced. (The increase, however, is smaller than that contributed by the addition of calcium itself, which is consistent with the lesser efficacy of barium in effecting secretion.) The experiment using sucrose (Fig. 7*B*) also suggests that the light-scattering signal does not depend primarily on an alteration of extracellular space, since the effect of repetitive stimulation should be smaller under conditions in which the terminals are shrunken by the hypertonicity of the bath, but the light-scattering change was not diminished.

On the other hand, the S-wave transparency changes that seem to reflect rapid alterations in light scattering are dramatically affected by many of the same interventions that are known to alter the release of neuropeptides from these terminals. The dependence on stimulation frequency (Figs. 2 and 3) is in good accord with evidence obtained in several laboratories suggesting that neuropeptide secretion increases with frequency of stimulation (Dreifuss et al., 1971; Nordmann and Dreifuss, 1972; Poulain and Wakerley, 1982; and unpublished data on the mouse neurohypophysis [H. Gainer et al., manuscript in preparation]), and the facilitation of the intrinsic optical response seen in virtually all of the records seems particularly telling. The effect of extracellular calcium concentration on the magnitude of the S-wave of the intrinsic optical signal (Figs. 4 and 5) is also in remarkably good agreement with the classical behavior of secretory systems (Douglas, 1978). The effect of replacing all of the H₂O in the Ringer's solution by D₂O, however, has not been reported previously in the mammalian neurohypophysis. Under these conditions, we found (Fig. 6) a

depression of the light-scattering signal of ~60%. Measurements of secreted vasopressin carried out in one of our laboratories (H. Gainer et al., manuscript in preparation), using radioimmunoassays, have revealed a nearly identical decrease in vasopressin release from the mouse neural lobes in D₂O under comparable stimulation conditions.

Another series of experiments, carried out in our laboratory together with T. D. Parsons, has shown that neomycin and other aminoglycoside antibiotics depress the S-wave (Parsons et al., 1985). This finding is significant because, at low concentrations, these drugs act presynaptically at the snake neuromuscular junction and depress evoked transmitter release by competing with $[Ca^{2+}]_o$ (Fiekers, 1983). Our observation that, for example, neomycin at 220 μ M concentration in normal Ringer's solution depressed the magnitude of the S-wave by 60% after 11 min provides additional evidence that these agents do act presynaptically, since there are no postsynaptic elements in the neurohypophysis, and that they act as competitive antagonists of $[Ca^{2+}]_o$ (Parsons et al., 1985). Further, the compatibility of the aminoglycoside effects on the light-scattering changes in the neurohypophysis, with voltage-clamp data reported from the neuromuscular junction (Fiekers, 1983), tends to support the interpretation that the S-wave reflects processes closely related to neuropeptide release.

We have, as yet, no reason to implicate any particular step, among the sequence of events that couples excitation to secretion, in the generation of the large intrinsic optical signals reported here, and the identity of the physiological event or events responsible remains unclear. The fusion of secretory vesicles during exocytosis should result in the loss of relatively high refractive index particles and thereby reduce the refractive index gradients in the terminal. However, the very early onset of the S-wave suggests that the optical signal could arise as a result of some calcium-dependent process prior to the fusion of secretory vesicles and the release of their contents. For example, it is possible that the light-scattering changes reported here reflect a phase transition of the contents of the secretory vesicles, or they may reveal rapid alterations in intracellular calcium stores after calcium entry but before secretion (Neering and McBurney, 1984). Thus, the intrinsic optical changes reported here may be related to the transparency changes observed in cut skeletal muscle fibers (Kovacs and Schneider, 1977; Rios et al., 1983) that precede tension development. The identification of the origin of the intrinsic optical changes must await precise measurement of light scattering per se. Laser light-scattering experiments, particularly in simplified model systems (e.g., secretosome and vesicle suspensions), should assist in the interpretation of the intrinsic optical signals. In particular, optical heterodyning and homodyning techniques (Earnshaw and Steer, 1983) using photon correlation spectroscopy should provide important information about the identity of the scatterers. In any event, the weak dependence on wavelength of the signal reflecting secretion, contrasted with the strong wavelength dependence of the extrinsic absorption signal provided by linear potentiometric probes (Ross et al., 1974; Cohen and Salzberg, 1978; Salzberg, 1983) (e.g., merocyanines), should permit one to monitor simultaneously, in a stained preparation, the voltage changes in the nerve terminals and the time course of events intimately associated

with the release of secretory products. The inherently fast responses of the two optical measurements might then improve our ability to resolve early events in the coupling of excitation to secretion.

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