Long-Lasting Intrinsic Optical Changes Observed in the Neurointermediate Lobe of the Mouse Pituitary Reflect Volume Changes in Cells of the Pars Intermedia

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Key Words

Chloride movement • GABA_A receptor • Light scattering • Neurohypophysis • Neurointermediate lobe • Pituitary • Volume changes

Abstract

Background/Aims: Complex intrinsic optical changes (light scattering) are readily observed in the neurointermediate lobe of the mouse pituitary gland following electrical stimulation of the infundibular stalk. Our laboratory has previously identified three distinct phases within the light scattering signal: two rapid responses to action potential stimulation and a long duration recovery. The rapid light scattering signals, restricted to the neurohypophysial portion (posterior pituitary) of the neurointermediate lobe, consist of an E-wave and an S-wave that reflect excitation and secretion, respectively. The E-wave has the approximate shape of the action potential and includes voltage- and current-related components and is independent of Ca²⁺ entry. The S-wave is related to Ca²⁺ entry and exocytosis. The slow recovery phase of the light scattering signal, which we designated the R-wave, is less well characterized. **Methods:** Using high temporal resolution light scattering measurements, we monitored intrinsic optical changes in the neurointermediate lobe of the mouse pituitary gland. Pharmacological inter-

ventions during the measurements were employed. **Results:** The data presented here provide optical and pharmacological evidence suggesting that the R-wave, which comprises signals from the posterior pituitary as well as from the pars intermedia, mirrors volume changes in pars intermedia cells following a train of stimuli applied to the infundibular stalk. These volume changes were blocked by the GABA-receptor antagonists bicuculline and picrotoxin, and were mimicked by direct application of GABA in the absence of electrical stimulation. **Conclusions:** These results emphasize the importance of central GABAergic projections into the neurointermediate lobe, and the potential role of GABA in effecting hormone release from the pars intermedia.

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Introduction

It is generally accepted that Ca²⁺-mediated secretion of the peptide hormones vasopressin and oxytocin from the neurohypophysis is accompanied by cell surface and volume changes associated with the fusion of secretory vesicles (dense-core granules) with the plasma membrane [1, 2], and the release of their contents. There is also evidence [3] for the convective flow of water during this process. However, exocytosis (and endocytosis) are rapid

processes [1, 4], whereas swelling that may be due, for example, to GABA-mediated increases in membrane transport, takes minutes [5]. All of these processes correlate with changes in the volume [6] and/or refractive index of cells and can thus be recorded as long-term changes in the intrinsic optical properties of whole tissue, as in the case of the mouse neurohypophysis [7]. While a change in refractive index could account, in principle, for changes in the intrinsic optical properties of tissues [8], in most instances volume changes are responsible [9]. Using a modified (dynamic high-bandwidth) atomic force microscope, we recently demonstrated that the rapid changes in light scattering (E-wave and S-wave) [7] exhibited by the mouse neurohypophysis are, indeed, associated with volume changes in that tissue [10]. The light scattering changes associated with exocytosis from mammalian nerve terminals were originally observed in a preparation of mouse pituitary (fig. 1a) in which the anterior pituitary (pars distalis) was separated from the neurointermediate lobe [neurohypophysis, also known as pars nervosa or posterior pituitary (PP)] and pars intermedia (PI) [7]. In these experiments, the axons passing through the infundibular stalk of the pituitary were stimulated electrically and the resulting changes in transmitted light through the neurointermediate lobe were recorded as light scattering signals. These intrinsic optical changes in the mammalian neurohypophysis are reliable tools for the study of excitation-secretion coupling. They can be recorded from an intact preparation under near-physiological stimulation conditions, providing an opportunity to observe, in real time, processes associated with hormone release, and to alter these processes by means of pharmacological agents.

The changes in light scattering previously observed [7, 11] in the neurointermediate lobe of the mouse (and other rodents) include at least three phases: two rapid light scattering signals consisting of an E-wave and an S-wave [7] (fig. 1b) that reflect excitation and secretion, respectively, and rise within milliseconds of the arrival of individual action potentials, and a long duration recovery (R-wave) lasting seconds to minutes (fig. 1c).

The E-wave includes voltage- and current-related components and is independent of Ca^{2+} entry, while the S-wave is related to Ca^{2+} entry and exocytosis. The E-wave is sensitive to specific ionic and pharmacological interventions that alter membrane voltage and current (e.g. changes in $[\text{Na}^+]_o$, $[\text{K}^+]_o$ and $[\text{Ca}^{2+}]_o$, and specific ion channel blockers, e.g. 4-AP or TTX). The S-wave, on the other hand, does not occur in the absence of the E-wave, and can be altered by experimental interventions known

Fig. 1. Light scattering changes recorded from the mouse neurointermediate lobe in response to trains of seven electrical stimuli ($500 \mu\text{s}$ at 16 Hz for 410 ms) applied to the infundibular stalk. **a** View of the mouse neurointermediate lobe in a dissecting microscope. Scale bar = 0.2 mm . **b** The intrinsic optical signal (light scattering change) recorded at 670 nm . The rapid downstrokes (E-waves) reflect the arrival of the action potentials (and action currents) in the terminals of the neurohypophysis (PP), and the sustained upward deflections (S-waves, increased transparency) represent decreases in large-angle light scattering that are closely coupled to secretion of arginine vasopressin and oxytocin. **c** Another recording of the light scattering change in response to the same stimulus train as in **b**, but displayed on a contracted time base to show the long duration response (R-wave). In all the optical signals, an increase in transmitted light intensity (decreased light scattering) is plotted upward. All of the traces here and in the following figures were recorded without averaging using a single silicon PIN photodiode mounted behind an adjustable diaphragm in the image plane of a compound microscope.

to modulate neuropeptide secretion (e.g. Ba^{2+} substitution for Ca^{2+} , changes in $[\text{Ca}^{2+}]_o$, stimulation frequency, D_2O -substitution for H_2O and application of Ca^{2+} -channel blockers [7, 12, 13] and caffeine [14]). The E-wave, in particular, reflects the arrival of the action potential and associated action currents at the neurohypophysial terminals (and secretory varicosities [15]), and correlates with the fluorescence and absorption signals observed with voltage-sensitive dyes [12, 13, 16–18], with the rising edge of signals from Ca^{2+} -sensitive dyes [17, 19], and with volume changes (the ‘mechanical spike’) recorded with a high-bandwidth modified atomic force microscope [10]. In short, the E-wave of the light scattering signal constitutes a voltage- and current-related component exhibiting the general shape of the invading action potential [16], and is followed by the S-wave, a component that reflects the processes of Ca entry and exocytosis.

The slow recovery phase of the light scattering signal (the R-wave), however, is less well understood. Previous experiments [20] had shown that this recovery phase is extremely sensitive to chloride replacement (90%) by impermeant anions (e.g. gluconate and methylsulfonate), and to blocking of chloride transport with stilbene derivatives such as 4-acetamido-4-isothiocyanato-stilbene-2,2-disulfonic acid (SITS) and 4,4'-diisothiocyanato-stilbene-2,2'-di-sulfonic acid (DIDS), and suggested that the R-wave may reflect transmembrane chloride movements coupled to volume changes in the tissue. All of these signals exhibit some wavelength dependence, especially in the late phase (fig. 2), but are readily measured from 400 to 1,000 nm.

The mammalian pituitary gland is a complex tissue. Developmentally [21] the pituitary derives from two different sources and comprises histologically different tissues. The anterior lobe, together with the PI is an up-growth of ectoderm from the roof of the stomatodeum, while the PP is derived from the neuroectoderm. Although it is relatively simple to separate the anterior pituitary from the rest of the pituitary because of the intraglandular cleft, the embrace of the PP by the PI, in which it is nestled, is so intimate that it is impossible to completely separate them mechanically. Because the intrinsic optical changes were actually measured from the composite tissue that is the neurointermediate lobe [7], and because the fast and slow components of the signal are *pharmacologically* separable, it seemed important to attempt to disentangle the *anatomical* origins of the observed light scattering changes, and to analyze, as much as possible, this complex signal into components that could be assigned to either the nerve terminals of the PP or to the cell bodies of the PI.

Fig. 2. Wavelength dependence of the light scattering change recorded from the neurointermediate lobe (PP plus PI) in response to the same train of seven stimuli as in figure 1. Recordings obtained at representative wavelengths.

Here, we achieve a partial differentiation of the light scattering signal, and we characterize more fully its recovery phase. Our data demonstrate that the R-wave includes light scattering contributions from both PP and PI and its normal shape requires the anatomical integrity of the neurointermediate lobe. The PI component of the R-wave is determined by the response of these cells to GABA released upon stimulation of the infundibular stalk, and is eliminated by GABA_A-receptor blockers. These experiments provide optical and pharmacological evidence for a central GABAergic innervation of PI and they also suggest that light scattering measurements may be used to monitor processes associated with secretion and their response to experimental intervention.

Materials and Methods

The neurointermediate lobe was obtained from CD-1 mice in the following manner. Females, 30–40 days old, were sacrificed by guillotine and exsanguinated. The head was pinned to the bottom of a Sylgard-lined dissection dish and its skin was removed from the skull. The skull was then opened along the dorsal midline and the upper portion removed bilaterally. The brain was reflected caudally and removed, after cutting optic and olfactory nerves under a low-power dissecting microscope. During this procedure, the infundibular stalk is automatically ruptured, leaving an infundibular stump and the entire pituitary gland in the base of the skull, held in place by thin connective tissue. Oxygenated mouse 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) was circulated over the pituitary, during the removal of the gland

Fig. 3. The effect of impermeant anion (isethionate) substitution for chloride in the bathing solution on the light scattering change recorded from the neurointermediate lobe (PP plus PI). Signals recorded in normal mouse Ringer's solution (NMR) before and after isethionate substitution are shown in black. The red trace exhibits the response to the same stimuli as in figure 1 when 93% of the chloride in the bathing solution was replaced with isethionate.

using iridectomy scissors and fine forceps. Once the pituitary was isolated, the anterior pituitary (pars distalis) could be separated from the neurointermediate lobe, comprising the neurohypophysis (pars nervosa) plus the PI. The PI consists of a delicate lacework of tissue surrounding the neurohypophysis and provides a convenient site for pinning to the bottom of an experimental chamber while preserving the integrity of the neurohypophysis.

The neurointermediate lobe was pinned down (with the neurohypophysis facing upward) on the thin Sylgard bottom of a simple chamber so that the infundibular stalk could be clasped between a pair of Pt-Ir (90%–10%) electrodes coated with Teflon except where they contact the infundibulum. Brief shocks (80–100 V), lasting between 300 and 500 μ s, were delivered through a stimulus isolator, and the resulting changes in the transparency of the neurointermediate lobe were recorded [7] using a large area PIN silicon photodiode (PV-444, Perkin Elmer Optoelectronics, Vaudreuil, Qué., Canada) and a current-to-voltage converter having a 1-M Ω feedback resistor together with a 500 $\times$ second stage amplifier. Illumination was provided by either a high power 740-nm LED (Roithner LaserTechnik, Vienna, Austria) [22, 23] or a 100-W tungsten-halogen lamp with a 675 ± 25 nm (Chroma Technology, Brattleboro, Vt., USA) filter. The photodiode was positioned in the image plane of a Zeiss UEM upright microscope behind an adjustable diaphragm, or mask, so that only light passing through the preparation was monitored. Unless otherwise noted, the objective was a 20×0.5 n.a. HCX APO water immersion lens (Leica, Solms, Germany). With adequate oxygenation, the resulting intrinsic optical signals can easily be recorded for several hours.

In all of the optical records, the ordinate scale represents changes in transmitted light intensity plotted in arbitrary units. The aggregate $\Delta I/I$ during the seven stimuli was typically $\sim 5 \times 10^{-3}$. The plots shown are representative of 5–20 replicate experiments.

Results

Figure 3 illustrates an experiment that is consistent with the outcome of some early experiments published only in abstract form [20] which we interpreted as demonstrating a direct link between Cl^- movements and the R-wave of the light scattering signal. These results, together with the presence of central GABAergic innervation of the mouse neurointermediate lobe [24–29], and the presence of GABA_A and GABA_B receptors on cells of the PI [30], led us to explore whether or not the R-wave was monitoring the physiological effects of GABA released when the infundibular stalk is stimulated electrically. Indeed, figure 4 demonstrates that the R-wave is sensitive to the GABA_A-receptor blockers bicuculline (100 μ M; fig. 4a) and picrotoxin (100 μ M; fig. 4b).

These experiments show that the effect of bicuculline is fully reversible while that of picrotoxin is not. Furthermore, the results in figure 4 extend our earlier observations [20] by demonstrating that this late component of the light scattering signal monitors, directly or indirectly, Cl^- movement through GABA_A-receptor channels.

If Cl^- movement is responsible for shaping the R-wave, we would expect that blocking Cl^- channels would have a similar effect on the R-wave as that of the GABA_A-receptor blockers. We, therefore, examined the effect of R(+)-IAA-94, a potent inhibitor of skeletal muscle and epithelial Cl^- channels, on the R-wave. Figure 5 demonstrates that, at 100 μ M concentration, R(+)-IAA-94 does, indeed, mimic the effect of bicuculline and picrotoxin on the recovery phase of the light scattering signal.

While GABAergic terminals and GABA_A-receptors are present in *rat* PP, and GABA itself inhibits neurosecretion in the neurohypophysis [27, 29], we find no evidence for any effect of GABA_A-antagonists on the component of the light scattering signal (the S-wave) that is related to secretion of the hormones oxytocin and arginine vasopressin by the terminals of the *mouse* PP (neurohypophysis). Indeed, figures 4c and 4d illustrate the complete absence of any effect of bicuculline and picrotoxin on the S-wave (or the E-wave) of the light scattering signal in the mouse neurohypophysis.

The PP is electrically excitable [31], and when stained with voltage-sensitive dyes [12, 13, 16–18] exhibits fast extrinsic optical signals that are readily identified as action potentials. PI cells, on the other hand, although capable of generating action potentials [32], are not directly excited by stimulation of the infundibular stalk and neither the extrinsic optical signal (not shown) nor the light scattering signal from PI tissue exhibits a rapid response fol-

Fig. 4. GABA_A-receptor blockers modify the light scattering signal recorded from the neurointermediate lobe. **a** 100 μ M bicuculline modifies the shape of the R-wave of the light scattering signal recorded from the neurointermediate lobe in response to the trains of seven electrical stimuli (500 μ s at 16 Hz) applied to the infundibular stalk. Black trace: light scattering signal recorded in normal mouse Ringer's solution (NMR) before application of bicuculline. Red trace: light scattering signal after 40 min in 100 μ M bicuculline. Green trace: light scattering signal recorded 44 min

after return to NMR. **b** 100 μ M picrotoxin also modifies the shape of the R-wave of the light scattering signal recorded from the neurointermediate lobe in response to the trains of seven electrical stimuli (500 ms at 16 Hz) applied to the infundibular stalk. Black trace: light scattering signals recorded in NMR before adding picrotoxin; red trace recorded after 65 min in 100 μ M picrotoxin; green trace recorded 30 min after return to NMR. Unlike bicuculline, the effects of picrotoxin were irreversible. **c** and **d** are expanded versions of the traces in **a** and **b**.

lowing action potential stimulation (see fig. 8, green trace). Despite the differences in electrical excitability of PP and PI, the light scattering signal that we observe in response to electrical stimulation applied to the axons that compose the infundibular stalk is actually a combination of the intrinsic optical change generated in the neural (PP) and intermediate (PI) lobes of the pituitary. In order to separate the observed light scattering change into components having identifiable anatomical origins, we attempted to dissect away as much as possible of the PI from the PP.

Although a complete separation of the PI is difficult because it carries the risk of compromising the integrity of the neurohypophysis, we were able to obtain interesting results following manual separation of the PI. Some of these results are shown in figures 6 and 7.

Figure 6 illustrates the consistent observation that, upon surgical removal of most of the PI, the effects of bicuculline (fig. 6a) and/or picrotoxin (fig. 6b) on the R-wave of the light scattering signal are greatly diminished. Indeed, there is virtually no difference in the light scattering signal in the presence or absence of either GABA-

Fig. 5. The effect of a chloride channel blocker on the R-wave of the light scattering signal recorded from the neurointermediate lobe (PP plus PI). The black trace shows the light scattering signal recorded in normal mouse Ringer's solution (NMR) from the neurointermediate lobe in response to the same train of seven electrical stimuli applied to the infundibular stalk. The red trace illustrates the effect of 16-min exposure to the Cl^- channel blocker, R(+)-IAA-94, at $100 \mu\text{M}$. The effect is very similar to that of the GABA_A -receptor blockers, bicuculline and picrotoxin.

receptor blocker. These results strongly suggest that bicuculline and picrotoxin bind to GABA_A receptors found primarily on PI cells. The change in the profile of the R-wave upon removal of the PI is also noteworthy (compare, for example, the control curves in fig. 6 with those in fig. 3–5) as the resulting R-wave has its origin primarily in PP.

For separating the two signals, a non-invasive alternative to physically dividing the tissue involves using a high numerical aperture objective to monitor the light scattering signals coming from different areas, as well as from different depths within the neurointermediate lobe. Figure 7 shows one such experiment. Signals from the PI were obtained by recording transmitted light at the edge of the preparation, where the PP tissue is thin to non-existent, and by using a diaphragm in the image plane of the microscope to select a field of view containing exclusively what could be identified as PI tissue. In addition, signals could be monitored, at different depths of focus, from the central region of the neurointermediate lobe, but at increasing distances from the PI, which lay against the bottom of the chamber. The red trace was recorded from the whole tissue with an open diaphragm in the image plane of the microscope. The black trace was recorded from the upper-center of the neurointermediate lobe, where the scattering is dominated by neurohypophysial

Fig. 6. With the surgical removal of most of the PI, the effects of bicuculline (**a**) and picrotoxin (**b**) on the R-wave of the light scattering signal are greatly diminished. **a** Black trace: PP in normal mouse Ringer's solution (NMR); red trace: 90 min in $100 \mu\text{M}$ bicuculline. **b** Black trace: PP in NMR; red trace: 90 min in $100 \mu\text{M}$ picrotoxin.

terminals (and the dense core secretory granules contained within them). The green trace was recorded from the edge of the neurointermediate lobe, where only the PI lies in the light path. Note that this trace completely lacks the fast light scattering components (E-wave and S-wave).

Using the same technique employed in figure 7, we found additional evidence for the conclusion that the GABA receptors responsible for shaping the downward deflection of the R-wave are confined to the PI. This is illustrated in figure 8. In this experiment, application of bicuculline ($100 \mu\text{M}$; red trace) completely abolished the light scattering signal having its origin in the PI (lower black trace), but had little or no effect (green trace) on the

Fig. 7. Optical separation of light scattering signals from the neurointermediate lobe in response to the same train of seven electrical stimuli as in figure 1 applied to the infundibular stalk. Red trace: recording from the whole tissue with open diaphragm in the image plane of the microscope; black trace: recording from the central region of the neurointermediate lobe, but with the focus on the PP where the scattering is dominated by the neurohypophysial terminals; green trace: recording from the edge of the neurointermediate lobe, where only the PI lies in the light path. In this case, there is no fast light scattering signal. All traces were recorded from the same preparation.

Fig. 8. The effects of bicuculline are limited to the light scattering changes recorded from the PI. Black trace: light scattering signals from the PP recorded in normal mouse Ringer's solution (NMR) in response to the same train of seven electrical stimuli applied to the infundibular stalk; green trace: the light scattering changes recorded from the PP after 40 min in 100 μ M bicuculline. The blue trace shows the light scattering signal recorded from the PI in NMR in response to the same train of seven electrical stimuli applied to the infundibular stalk; the red trace illustrates the response in the PI after 40 min in 100 μ M bicuculline.

light scattering signal originating in the neurohypophysis (PP, upper black trace). The same results were obtained using 100 μ M picrotoxin (data not shown).

The results presented above implicated GABA as a potent modulator of the R-wave of the light-scattering signal and identify the PI as the locus where the most relevant GABA receptors are expressed. However, the evidence presented thus far relied on GABA released upon electrical stimulation of the infundibular stalk, and cannot preclude synergistic interactions between GABA and other neuropeptides or neurotransmitters, either co-secreted or released from nearby terminals in response to the same electrical input.

To examine unambiguously whether GABA itself has a direct effect on the PI, we monitored the light-scattering changes exclusively from the PI portion of the neurointermediate lobe during direct bath application of the neurotransmitter. The preparation exhibited a dose-dependent response to GABA application at concentrations ranging from 0.1 to 4 mM. Figure 9 shows the result for 4 mM. The black trace illustrates the intrinsic optical signal recorded from PI while the neurointermediate lobe was superfused with normal mouse Ringer's solution

Fig. 9. The light scattering changes recorded from the PI in response to bath application of GABA. Black trace: response to bath application of normal mouse Ringer's solution (NMR; control); red trace: response to bath application of Ringer's solution containing 4 mM GABA.

Fig. 10. Changes in light scattering in response to acute changes in bath osmolarity. **a** Transient decrease in transmitted light intensity (increase in light scattering) in response to a Ringer's solution made 10% hypertonic with sucrose. **b** Transient increase in transmitted light intensity (decrease in light scattering) in response to application of a Ringer's solution made hypotonic by the addition of 20% distilled H₂O.

(NMR). The red trace shows the intrinsic signal recorded from the same spot while the tissue was exposed to a solution containing 4 mM GABA. Both traces were recorded in the absence of electrical stimulation. The intrinsic optical change in response to a direct application of GABA resembles qualitatively the records shown in figure 7 (green trace) and figure 8 (black traces), all of which exhibited the recovery phase (R-wave) of the light scattering signal from the PI following electrical stimulation of the infundibular stalk.

We have also observed that bath application of GABA produces the same effect on the optical properties of the

PI as does a solution made 10% hypertonic with sucrose, a solution that induces cell shrinkage. This is illustrated in figure 10.

Discussion

The hypothalamus is the portion of the brain where neural signals are converted to humoral ones. Secretion of arginine vasopressin and oxytocin by the nerve terminals and axonal varicosities of the neurohypophysis (PP) has been studied extensively as a function of electrical activity in the magnocellular neurons of the hypothalamus. The role of GABA in hormone release, however, is less well understood. GABA appears to act as a regulatory factor in the control of hormone secretion in different parts of the pituitary gland. The control mechanisms involve GABAergic terminals of the PP as well as GABA_A and GABA_B receptors of the PP and PI. Neuronal control of pituitary function by GABA via direct innervation of secretory cells of the PI by the axon terminals of hypothalamic neurons was demonstrated by immunodetection of GABA [33, 34], by intracellular recordings from the PI in response to both pituitary stalk stimulation and exogenous GABA application [26, 28], by immunohistochemical studies using antibodies to the enzyme responsible for production of GABA, viz., glutamate decarboxylase [25, 27], by measurement of glutamate decarboxylase activity [24], and by immunohistochemical localization of the GABA stores in nerve terminals using antibodies to the vesicular GABA transporter [35]. Although GABA_A receptors are present in both the PP and in the PI [33, 34], the GABA-activated currents in the PI are much larger [36], and this can readily account for the result that the R-wave is seen predominantly in the PI. Our measurements of intrinsic optical changes in the neurointermediate lobe of the mouse pituitary gland add to the current understanding of the role of central GABAergic innervation of the pituitary.

GABA appears to cause the shrinkage of neurons in embryonic brainstem slices [9], if the reported changes in light scattering are correctly interpreted as changes in extracellular volume [8]. Using dynamic high-bandwidth atomic force microscopy, our laboratory recently demonstrated that light scattering changes in the electrically stimulated PP are associated with volume changes in neurohypophysial terminals [10]. To establish the correlation between light scattering in the PI and the possible shrinkage/swelling of tissue provoked by bath application of GABA (fig. 9) we recorded light scattering signals in

response to acutely altered osmolarity of the bathing solution (fig. 10a, b). Figure 10a illustrates the transient decrease in transmitted light intensity when the PI is shrunken as the result of flowing a Ringer's solution rendered 10% hypertonic with sucrose. In contrast, figure 10b demonstrates the increased transparency associated with swelling of the PI resulting from changing the Ringer's solution bathing the neurointermediate lobe to one made hypotonic by the addition of 20% distilled H₂O to the Ringer's solution. From these measurements we can conclude that in PI tissue, GABA produces an intrinsic optical effect analogous to the effect of hypertonic solution – the transparency of the tissue decreases along with shrinkage. An alternative hypothesis, viz., that the light scattering change observed in the PI is related to Ca²⁺ entry and subsequent exocytosis and/or endocytosis, is not consistent with our observation that the light scattering change that follows bath application of GABA is not reduced in magnitude when Ca²⁺ entry is prevented by Cd²⁺ (0.5 mM).

Volume changes are intimately related to water homeostasis and are critical for normal brain functioning. For example, a K⁺ and Cl⁻ channel-mediated volume decrease occurs early, before the characteristic ultrastructural or biochemical events associated with apoptosis [37]. Moreover, cell volume decrease may be inhibited pharmacologically by blocking the K⁺ or Cl⁻ channels implicated in cellular volume regulation [38]. Further, the small volume changes associated with exocytosis in nerve terminals [2, 3] affect extracellular ion concentrations and, thence, volume. The volume regulation and ionic homeostasis contributed by plasma membrane Cl⁻ channels [39], including GABA_A receptors, is carried out in dynamic equilibrium with the operation of various transporters, with water movement mediated by aquaporins and by co-transporters that are expressed in the plasma membrane [40]. As already noted, we have observed

(fig. 9) that bath application of GABA produces the same effect on the optical properties of the PI as does a solution made 10% hypertonic with sucrose, a solution that induces cell shrinkage. The volume restoration that follows may be due to the turnover of the GAT-1 GABA-transporter [41]. Thus, we can speculate that if water secretion associated with exocytosis [3] and/or cell shrinkage due to direct GABAergic innervation does take place, the transporter (GAT-1) may supply the water that restores cell volume.

The light scattering measurements presented here allow us to conclude that: (1) the component of the long-term optical response (R-wave) that is sensitive to Cl⁻-ion movements reflects changes in the PI, and (2) the full time-course of the response takes minutes and is likely, therefore, to encompass not only Cl⁻ channel opening by GABA, but also some other processes that are responsible for restoration of the physiological Cl⁻ concentrations. Note that the R-wave frequently undershoots the baseline and that full recovery usually takes minutes. It is reasonable to suppose that the complex shape of the R-wave is partially determined by the GAT-1 transporter, which could account for its biphasic shape, but this remains to be clarified by future experiments.

The data presented here provide optical evidence for the idea that the recovery phase (R-wave) of the light scattering signal reflects events which are involved in modulating the process of hormone release by the PI, and also provide some optical and pharmacological arguments for a central GABAergic innervation of the pituitary, where GABA acts as a factor in hormone release.

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