Turbidity Change of the Mouse Adrenal Medulla Evoked by Acetylcholine Stimulation

Jin-Hong Fan* and Susumu Terakawa

Department of Cell Physiology, National Institute for Physiological Sciences, Okazaki, 444 Japan

Summary The turbidity of the mouse adrenal medulla was measured using a photodiode and a video system equipped with an image processor. Acetylcholine applied at concentrations of 30 to 100 µM induced a slow decrease in turbidity by about 0.1% of the resting value. This response was suppressed by Cd-containing or Ca-deficient medium. These results and a video image suggested a structural change in the chromaffin cells associated with the secretory activity.

Key words: chromaffin cell, acetylcholine, digital imaging.

Intracellular mechanisms underlying the externalization of granular contents of secretory cells may involve not only chain reactions of signaling molecules but also structural changes of internal organelles. Such structural changes in active secretory cells can be most directly elucidated by optical methods. Laser Doppler measurements, for example, suggested an increase in granular movements in neurosecretory terminals stimulated with K-rich solution (Englert and Edwards, 1977). Observations with the Nomarski microscope revealed a change in shape of isolated chromaffin cells upon stimulation with acetylcholine (Englert, 1980), and showed a vague image of exocytosis (Edwards et al., 1984). Laser light scattering measurements revealed that Mg-ATP induces swelling of secretory granules isolated from chromaffin cells (Warashina, 1985). We report here turbidity changes of the adrenal medulla probably associated with secretory activities. Since the disassembly of cytoskeletal fibers, swelling of secretory granules, and solubilization of granular contents are likely to influence the scattering power of secretory cells, real-time measurements of the turbidity of cells will give information about these structural changes of intracellular organelles.

The adrenal gland was excised from male mice (6–10 weeks, ddy strain). The
cortical layer was removed using fine forceps under a dissecting microscope. The isolated medulla (0.6 × 0.8 mm wide and 0.4 mm thick) was embedded in cotton wool and placed in a thin acrylic chamber. The chamber was placed under a bright field microscope (Labophot, Nikon, Tokyo) in such a way that a central part of the preparation could be observed through an objective lens of ×20 magnification. The preparation was then superfused with oxygenated Locke's solution containing (in mM): NaCl, 154; KCl, 5.6; CaCl₂, 2.0; MgCl₂, 1.0; glucose, 5.0; Tris-HCl, 10 (pH 7.4). A light from a tungsten-iodine lamp was passed through a heat suppression filter and a bandpass filter (400–700 nm), then condensed on the preparation. The light collected by an objective lens was split into two beams by a half mirror. One of the light beams was focused on a video camera (C-1800, Hamamatsu Photonics, Hamamatsu, Japan) using a relay lens (×1). The other beam was focused on a frame which limited the field of observation to be the same as that of the video camera. The intensity of the light passed through the frame was measured with a photodiode (S-1227, Hamamatsu Photonics), and recorded on a chart-recorder. Video signals were processed as described previously (TERAKAWA and NAGANO, 1987a). An image processor (PIP-4000, ADS Co., Osaka, Japan) was used to extract temporal differences between video images in real time. Firstly, a control image was stored in a frame memory at about 10 s before stimulation. Then, this image was subtracted from successive images, and results were continuously displayed with a digital contrast enhancement. The portion with decreased turbidity was thus displayed as a dark area in the processed image, and the portion with increased turbidity was displayed as a bright area (this relationship was opposite to that in the previous work (TERAKAWA and NAGANO, 1987a), but it was more convenient to reproduce small differences in photographic density). When the preparation showed a change in turbidity with some spatial differences, the monitor displayed a two-dimensional image consisting of bright and dark areas while the chart-recorder showed a total change in light intensity of the same field. All observations were performed at room temperature (24–28°C).

The intensity of light passed through the preparation increased gradually by about 10% in the first 40–60 min after the onset of perfusion with Locke's solution, and then it reached a steady level. At that moment, addition of acetylcholine (ACh) chloride to Locke's solution at a concentration of 30 µM induced a slow increase in light intensity by about 0.1% in 3 min (Fig. 1A). Addition of 100 µM ACh induced a similar response with a slightly rapid rising phase and a larger amplitude (Fig. 1B). When the ACh application was continued, the light intensity reached a peak in the initial 3 min and slowly fell down to a level slightly above the baseline (Fig. 1C). This low level was maintained until the medium was switched back to ACh-free Locke’s solution. The light intensity finally returned to the original baseline in 3 min in Locke’s solution. Figure 2 shows video frames obtained after the image processing for subtraction. An image obtained at a few seconds before the application of ACh was uniformly gray, indicating the absence of optical change during the control
An image obtained at 1.5 min after the onset of ACh application (time indicated by an arrow in Fig. 1 B) had many dark spots superposed on a dark background. Most probably, dark spots were responses in chromaffin cells because their shape and diameters (10–30 μm) were similar to those of chromaffin cells (COUPLAND, 1965). The dark background could also be responses in those cells which were out of focus. A non-uniform appearance of responding cells could be
explained by a similar distribution of catecholamine-containing cells identified by the fluorescence after a formaldehyde treatment. Since the preparation moved to some extent all the time, differential images recorded after a period longer than 2 min showed a characteristic pattern made of dark and bright areas aligned alternately in the direction of movement. Thus we could not follow the response of each cell for its whole process. Occasionally, these dark spots contrasted against a bright background. Appearance of this bright background suggested the presence of an additional source of response—probably an active movement of smooth muscles induced by ACh.

When ACh was applied to the preparation immersed in medium containing 50 µM Cd ions, these optical responses were suppressed completely (Fig. 3A). A similar suppression was obtained when the concentration of Ca ions was reduced to a level of 0.1 mM (Fig. 3B). To obtain these suppressing effects, the Cd-containing solution or the Ca-deficient solution had to be introduced 10 min before the application of ACh. After such a suppression, the response could be restored by switching the medium to the initial one, but its direction was frequently opposite to that of the original response. Recoveries with a direction the same as the original were observed in 5 preparations and recoveries with a differing direction were observed in 7 preparations. Initiation and termination of the responses of the

![Fig. 3. Suppression of the ACh-induced turbidity response. Top: control records. Middle: records obtained 10 min after addition of 50 µM CdCl₂ to the medium (A) and 10 min after reduction of the CaCl₂ concentration in the medium from 2.0 to 0.1 mM (B). Bottom: records obtained 15 min after switching the media back to initial solutions. ACh of 100 µM was applied during the period indicated by horizontal bars (4.5 min in A and 3 min in B).](image-url)
reversed sign were occasionally rapid to form a rectangular time course. These responses could be repeatedly suppressed further by Cd ions, but could not be reversed with its sign again even after 1-h wash. The video image of the reversed response consisted of many small dark spots in an irregularly shaped bright and dark background.

Release of catecholamine to the perfusion solution from the present preparation was confirmed using the electrochemical detector as described by Kumakura et al. (1988). The detector was connected directly to the outlet tubing from the chamber and the amperometry was performed at $-450\, \text{mV}$. Addition of 100 $\mu\text{M}$ ACh to the perfusion solution induced a release of catecholamine of about $9 \times 10^{-11}\, \text{mol}$ in 3 min. Both rise and fall of the release were faster than those of the turbidity change.

The results described suggest that ACh induces a decrease in turbidity in the adrenal medullary cells associated with catecholamine release. Because of the spotty pattern observed through the video system, the turbidity change is likely to reflect intracellular events for secretion of catecholamine in chromaffin cells which would produce a similar turbidity change in the neurohypophysis (Salzberg et al., 1985; Terakawa and Nagano, 1987a, b). Any inhomogeneity of refractive indices in the cells contributes to the cellular turbidity. However, the suppression of the response by Cd-containing solution or by Ca-deficient solution suggests only a few possibilities underlying the turbidity response of chromaffin cells. The most probable cellular change is the degranulation or exocytosis of secretory granules. This process involves a change in granular diameter (Pazoles and Pollard, 1978; Pollard et al., 1979; Warashina, 1985; Holz, 1986) and solubilization of granular contents which can cause great changes in local refractive indices. The ACh-induced turbidity response in chromaffin cells is slow and persistent in comparison with the time course of catecholamine release from chromaffin cells (our result, and Douglas and Rubin, 1961). A plausible explanation for this is that the turbidity response is related to the total amount of release and not the instantaneous rate of release. An accumulation of exocytotic changes in the cell may be the cause of the optical change. Such a structural change probably recovers slowly after the catecholamine release. An aggregation of granules mediated by linking proteins (Creutz et al., 1978; Pollard et al., 1979; Geisow and Burgoyne, 1983) and a disassembly of the cytoskeletal network (Cheek and Burgoyne, 1986; Lelkes et al., 1986; Perrin et al., 1987) may also somewhat contribute to the optical signal.

In addition to the response in chromaffin cells, some part of the responses may be ascribed to smooth muscles (Coupland, 1965) or other contractile elements. The response which appeared in large areas had occasionally an irregular shape and unpredictable polarities. Probably, smooth muscles (and endothelial cells) in blood vessels were stimulated by ACh and contracted in a complex way to produce responses of both polarities. The upward response could be an addition of responses from chromaffin cells and smooth muscles. A decrease in response of chromaffin cells with an unaltered response of smooth muscles could explain the reversal of the
polarity of responses after Cd application. Cellular movements evoked by ACh might be important for some sort of facilitation of catecholamine release from the adrenal medulla in vivo.

The results indicate structural changes in the chromaffin cells evoked by ACh. However, contractile movements of smooth muscles or some other cells hamper further analyses of such structural changes in the present preparation. This problem is now circumvented in our laboratory by observing optical changes in an isolated single chromaffin cell through a computer-enhanced video system.

We are grateful to the Uehara Memorial Foundation for providing financial support for J.-H. Fan’s stay in Japan.

REFERENCES


Japanese Journal of Physiology
OPTICAL RESPONSES IN ADRENAL MEDULLA


scattering accompany secretion by nerve terminals in the mammalian

TERAKAWA, S. and NAGANO, M. (1987a) Visualization of secretory activities in the Xenopus

TERAKAWA, S. and NAGANO, M. (1987b) Functional differentiation in the frog neuro-

WARASHINA, A. (1985) Changes in the size of isolated chromaffin granules in ATP-evoked