Single-Cell Contraction Assay for Human Ciliary Muscle Cells

Effect of Carbachol

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Purpose. The authors developed an assay to observe the contraction of a single human ciliary muscle cell.

Methods. Cultured human ciliary muscle cells were partially detached from the culture dish by incubation with a nonenzymatic dissociation buffer and treated with carbachol or pilocarpine. Contraction was quantified by measuring the cross-sectional surface areas of the cells.

Results. Carbachol decreased the cell surface area in a time-dependent manner. Contraction was observed within 1 min after the addition of carbachol and completed in less than 15 min. The effect of carbachol was dose dependent. For example, at 10 min after treatment with 10 μmol/l carbachol, the relative surface areas of cells decreased to 47% ± 4% (mean ± standard error of the mean, n = 7, with surface area at 0 min defined as 100%). The relative surface areas were 74% ± 4% (n = 7) after 1 μmol/l and 100% ± 9% (n = 7) after 0.1 μmol/l carbachol treatment. This contractile effect was antagonized by pretreatment with atropine, a specific muscarinic antagonist.

Conclusions. A simple method was established to study the functional changes of human ciliary muscle cells. Invest Ophthalmol Vis Sci. 1993;34:1876–1879.

Muscarinic agonists, such as pilocarpine, have been used in the treatment of glaucoma for more than 100 yr. These agents modulate accommodation and increase aqueous outflow of the eye.[1,2] It is generally accepted that their actions are mediated by the contraction of the ciliary muscle.[3,4] However, little is known currently about the cellular physiology and function of this muscle. Because of its inaccessibility, in vivo studies are extremely challenging.[2–4] In vitro contraction experiments using isolated ciliary muscles are similarly difficult because both the dissection and the monitoring of changes are laborious.[5,6] During recent years, cell culture has become a powerful research tool to investigate the functional properties of many tissues at a cellular level. We report here the development of an in vitro assay to observe and quantify the contraction of individual human ciliary muscle cells.

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METHODS. The isolation and culture of human ciliary muscle cells were performed essentially as described earlier.[7] The cells were subcultured by trypsinization and adapted to growing in Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, NY), supplemented with 4 mmol/l of L-glutamine (Sigma, St. Louis, MO) and 10% fetal calf serum (Hyclone, Logan, UT), at 37°C with 5% CO_2. In this report, cells of passages eight to ten were used. On the day of the study, they were partially detached from the plastic culture dish by replacing the medium with a nonenzymatic cell dissociation buffer (catalog no. C-5914, Sigma) and incubated at 37°C for 30–40 min. Carbachol (Sigma) was then added. When indicated, atropine (Sigma) was added 5 min before the addition of carbachol. Photomicrographs were taken at time intervals throughout the experiment. The cross-sectional surface areas of the cells were obtained by projecting the cell images of each photograph through a video camera onto a monitor screen of a personal computer. The cell images were then manually outlined, and the surface areas enclosed by the outlines were quantified using image-analysis software (BioQuant). A decrease in cell cross-sectional surface area was interpreted as an indication of cell contraction. To normalize the changes, the relative areas of the cells were used. The relative area of a cell was defined as the surface area at the indicated time point divided by the surface area at time 0 (10 sec before the addition of agonist) of the same cell.

RESULTS. Figure 1 illustrates that, 5 and 10 min after the addition of 10 μmol/l of carbachol, there was a dramatic decrease in the surface area of most cells. No significant change in cell area was observed during the 5-min period before the drug was added. This change in cell surface area was interpreted as an indication of contraction, rather than a decrease in cell volume, because treating the cells with hyperosmotic medium (supplemented by 200 mmol/l NaCl) did not induce surface area shrinkage. Instead, the cells flattened (data not shown). The cellular contraction was obvious within 1 min of carbachol treatment (Fig. 2), and the surface area stabilized at approximately 10 min after the application of the muscarinic agonist. This carbachol-induced contraction was dose dependent. As demonstrated in Figure 3A, 0.1 μmol/l of carbachol was ineffective in producing contraction of the muscle cells, whereas 1 μmol/l caused a partial contraction (ie, the area of the cells decreased to 70% of the initial surface area). Higher concentrations of carbachol induced additional contraction of the cells: 10 and 100 μmol/l of the agonist reduced the cells to 40–50% of their original sizes. A dose–response curve was then constructed by plotting the relative areas of the cells 10 min after carbachol treatment versus the

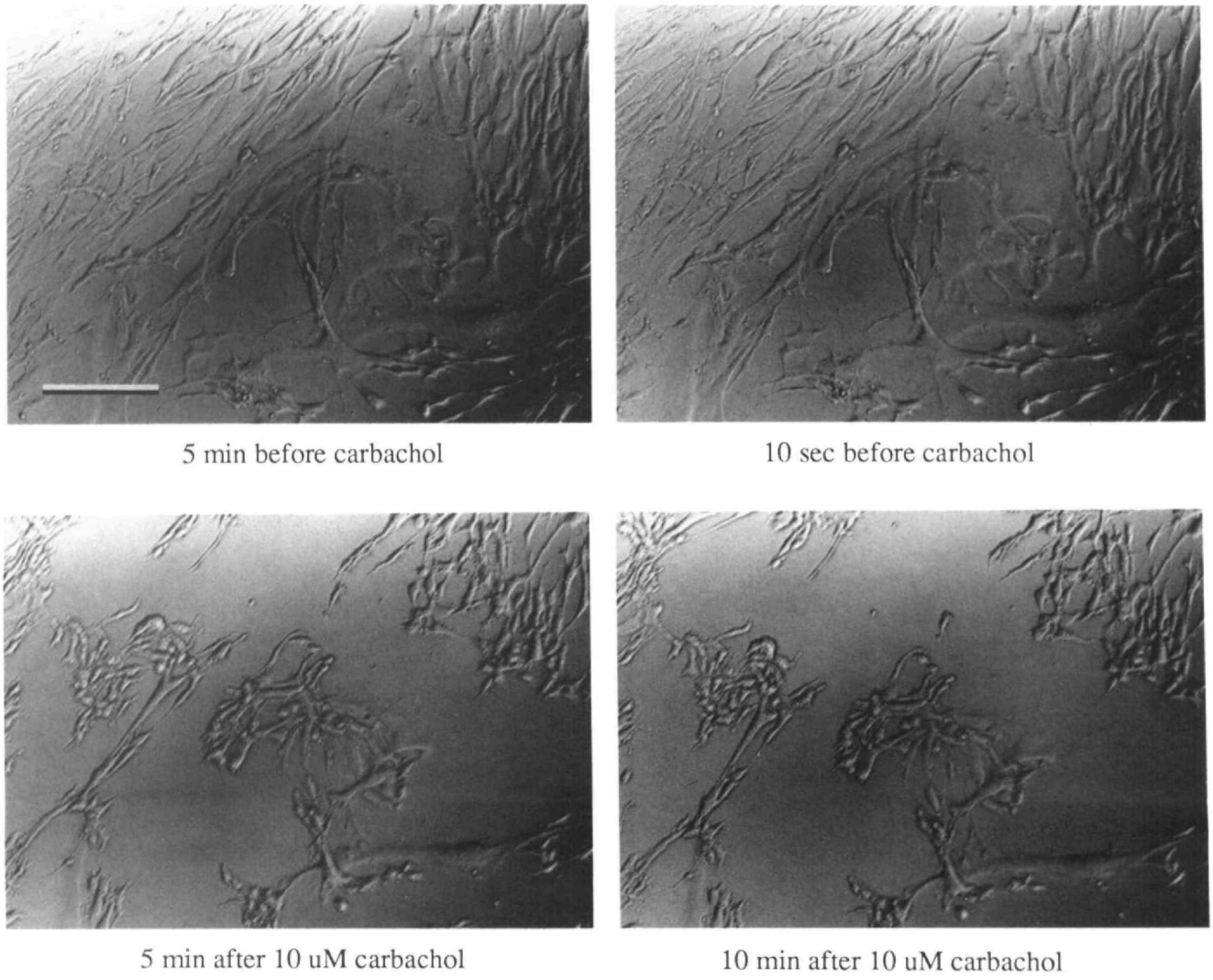

FIGURE 1. Photomicrographs of human ciliary muscle cells during contraction induced by carbachol. The cells were partially detached from the culture dish by pretreatment with cell dissociation buffer. Carbachol (final concentration, 10 μmol/l) was added 30 min later. Photomicrographs were taken at various times. Images of the cells at 5 min or 10 sec before carbachol addition and 5 or 10 min after carbachol addition are shown here. It is clear that, before carbachol treatment, there was a negligible change in the cell shapes, whereas the cross-sectional surface areas of most cells decreased significantly after exposure to carbachol. Bar represents 200 μm.

concentration of carbachol used (Fig. 3B). The effective dose of carbachol for 50% of the maximal effect was estimated to be 1–3 μmol/l.

The carbachol-induced contraction was apparently mediated by muscarinic cholinergic receptors. Pilocarpine, another muscarinic agonist and well-known ocular hypotensive compound, also caused the isolated ciliary muscle cells to contract (relative area at 10 min, 60 ± 5% [mean ± standard error of the mean, n = 7]) after 0.1 mM pilocarpine treatment). Furthermore, the effect of 1 mmo/l carbachol was completely blocked by pretreatment of the cells with 1 μmol/l of atropine, a muscarinic antagonist (data not shown).

DISCUSSION. We report here a unique in vitro assay for monitoring the contraction of individual human ciliary muscle cells. Under the conditions used, we showed that muscarinic agonists, carbachol and pilocarpine, caused the muscle cells to contract in a dose-dependent, time-dependent, and atropine-reversible manner. These results, which agree with previous in vivo[1] and in vitro[5,6] findings that muscarinic agents induce contraction of the ciliary muscles, validate the single-cell contraction assay as a meaningful model for the study of these muscles. This assay offers many advantages. For example, after the initial establishment of cell culture, the assay is relatively simple

and convenient. All the necessary equipment is commercially available, and the procedures can be carried out in a relatively short period. Each study requires few cells, which suits the cell's slow growth. Furthermore, because the change in cell area after drug treatment is sufficiently fast, it is usually immediately obvious by visual observation under the microscope. Thus, qualitative results are obtained promptly, which should be helpful in guiding the design of subsequent experiments. Compared with studies using isolated tissues containing many cell types, the single-cell assay is carried out on a homogeneous population of cells. Therefore, it is easier to correlate the functional physiologic changes of the cells with the biochemical changes observed in separate experiments. Moreover, the ciliary muscle cells are relatively large, which allows manipulation of their intracellular environment by microinjection of various molecules. Changes of cell behavior can then be studied. Nevertheless, this assay is not free of drawbacks. The contraction of cells occurred only after they were partially detached from the culture dish by pretreatment of a calcium-free nonenzymatic dissociation buffer. This limits the use of the assay to contractions that depend exclusively on influxes of extracellular calcium. We have tried to culture the cells on various matrices, such as Agarose (Sigma, St. Louis, MO), collagen, fibronectin, Matrigel (Collaborative Biomedical Products, Bedford, MA), or human extracellular matrix, with the rationale that the interaction between the cells and certain matrices may allow contraction without prior partial detachment. It was found that either the cells did not attach to the matrix or the matrix did not help the observation.

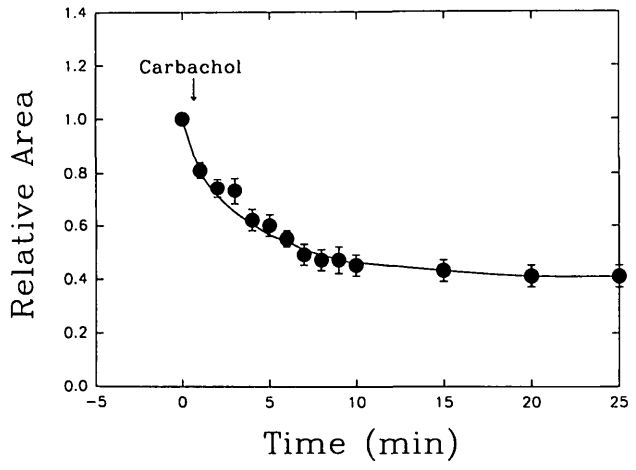

FIGURE 2. The time course of the carbachol-induced decrease in cell surface area. Symbols represent the mean ± the standard error of the mean of the relative areas of ten cells at different time points after carbachol (1 mmol/l) treatment. The relative area is defined as the surface area of a cell at the indicated time divided by its surface area at time 0 (10 sec before the addition of carbachol).

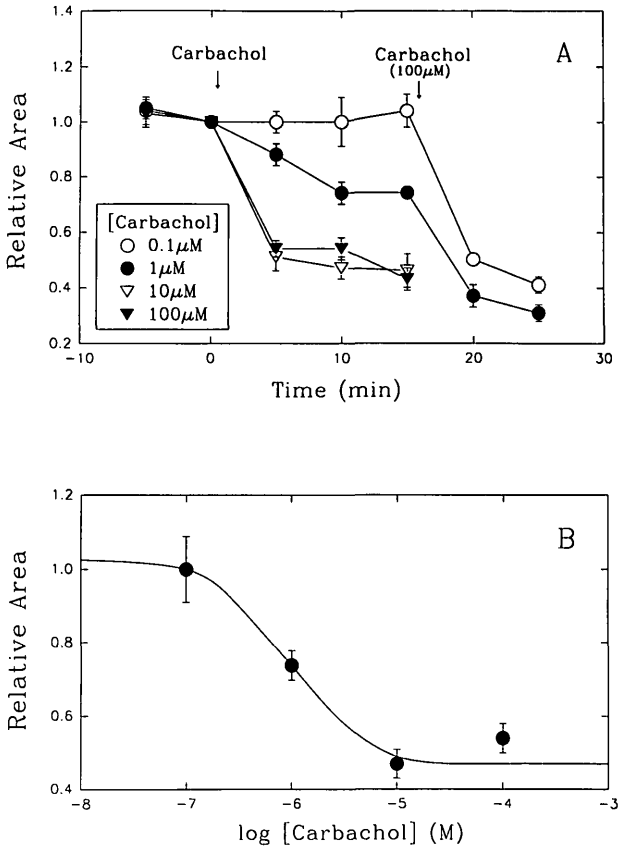

FIGURE 3. Effects of various doses of carbachol on ciliary muscle cell contraction. The ciliary muscle cells were treated with 0.1–100 μmol/l of carbachol, and their surface areas were monitored. (**A**) Time courses of changes. Symbols represent the mean ± the standard error of the mean of seven cells at each dose. At 15 min, carbachol (100 μmol/l) was added to some of the samples to demonstrate their full contraction capability. (**B**) Dose–response curve of carbachol. The relative areas 10 min after carbachol treatment are presented here. Symbols represent the mean ± the standard error of the mean of seven cells.

Thus, we developed a simple and expedient method to study the functional changes of human ciliary muscles cells. The validity and usefulness of this assay are demonstrated by the results of muscarinic agonists. With the many advantages stated previously, this method should provide a novel approach in the pursuit of understanding ciliary muscle cell biology.

Key Words

ciliary muscle cell, single-cell contraction assay, carbachol, pilocarpine, atropine

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Whatever Happened to Abstracts From Different Sections of the Association for Research in Vision and Ophthalmology?

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Purpose. The authors investigated the fate of abstracts from each ARVO section (May 1985 meeting), the overall publication percentage, and the journals in which the abstract-derived articles were published.

Methods. They performed a MEDLINE search by first author for 25 or 26 randomly selected abstracts from each section to identify those that led to full-length articles in peer-reviewed journals.

Results. Overall, 63% of abstracts led to full-length articles in peer-reviewed journals within our search period of 87 months. The publication rate of oral presentation abstracts (68%) was significantly higher than that of poster presentation abstracts (56%). A greater proportion of basic science-oriented abstracts (67%) led to publication than the clinically oriented abstracts (56%). The rate of publication was lowest for the Cornea section (40%) and highest for Physiology and Pharmacology (80%) and Biochemistry (76%). The abstract-derived articles were published in 67 different peer-reviewed journals, with 43% of the articles appearing in only five journals.

Conclusions. The fact that the majority of abstracts led to full-length articles supports ARVO's goal of a large interdisciplinary appeal with the exchange of ideas among different investigators. Invest Ophthalmol Vis Sci. 1993;34:1879–1882.

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We previously reported that overall 57% of abstracts presented at the annual meetings of ARVO and 60% at the American Academy of Ophthalmology ultimately led to full-length articles in peer-reviewed journals.[1] However, our previous study, because of a relatively small sample size (100 abstracts), was not powerful enough to allow us to investigate the rate of publication for each section of ARVO or the specific peer-reviewed journals in which articles were published. The current study was undertaken, with a much greater sample size and longer follow-up period, to determine the rate of publication of abstract-derived full-length articles for each section of ARVO, the overall publication rate, and the specific peer-reviewed journals in which these publications appeared.

METHODS. The abstracts of all the oral presentations and posters given at the May 1985 Annual Meeting of ARVO were identified in the Annual Meeting Abstract Issue. After assigning numbers 1–1693 to all these abstracts, we randomly selected for our analysis 25 or 26 abstracts from each section, totaling 327 abstracts for all sections. The random numbers were generated using Statworks (Cricket Graph, Malvern, PA) statistical software package. We conducted a literature search for abstract-derived articles as previously described.[1] Briefly, we conducted a search using the MEDLINE data base for all full-length articles published in peer-reviewed journals for the period from January 1984 through August 1992, using only the first authors of the abstracts. The MEDLINE search allows us to locate abstract-derived articles even if the abstract's first author became second, third, fourth, or last author of the final manuscript. We hypothesized that the person presenting the abstract, the first author, would be one of the authors of a formal full-length publication. In our previous study,[1] we performed a MEDLINE search by all the listed authors for abstracts not identified as leading to articles when