HOMOGENEOUS CELL POPULATIONS FROM RABBIT KIDNEY CORTEX

Proximal, Distal Tubule, and Renin-Active Cell Isolated by Free-Flow Electrophoresis

HANS-G. HEIDRICH and MARGARET E. DEW

From the Department Hannig, Max-Planck-Institut für Biochemie, D-8033 Martinsried b. München, Federal Republic of Germany

ABSTRACT

A single-cell suspension has been prepared from rabbit kidney cortex by using a Ca-binding medium and gentle mechanical forces. The suspension was subjected to carrier-free electrophoresis, and several cell fractions were obtained. Proximal and distal tubule cell populations could be identified by their morphology. Renin-containing cells were located by means of a radioimmunoassay. The morphology of the cells and their vitality (uridine incorporation) are discussed.

KEY WORDS kidney cells cortex renin separation electrophoresis

The successful isolation and separation of homogeneous cell populations from the kidney cortex in preparative quantities has not yet been described. However, cell suspensions have been produced by either mechanical or enzymatic treatment of the kidney or kidney slices (see, references for example, 3–5, 9, 10, 15, 25–27). Such preparations generally contain tubular elements as well as cells. The separation of a particular cell type from such cell suspensions is difficult or even impossible, since the classical techniques for particle separation are based on defined size differences of the particles to be separated (velocity sedimentation, 1 g) or on defined densities of the particles (density sedimentation). This means that for a successful application of these techniques it is necessary to have both a single-cell suspension and intact cells with defined density properties. These conditions have been achieved for hepatocytes in a number of laboratories, allowing a successful separation of different types of hepatocytes by density sedimentation (7, 28).

This paper presents a method for preparing single-cell suspensions from kidney cortex by using a Ca-binding medium. In addition, experiments are described in which proximal, distal tubule and renin-active cell populations are isolated from such suspensions by free-flow electrophoresis (14, 16, 17). The morphology and the vitality of the cells thus obtained are discussed.

MATERIALS AND METHODS

Isolation of Cells

One kidney was removed from a rabbit (New Zealand, white and black, 1,500–2,000 g) and immediately perfused through the arteria renalis with 70 ml of Earle’s balanced salt solution (supplemented with 1 g of glucose, 17.11 g of sucrose, 2.2 g of sodium bicarbonate, 13 ml of a 200-mM L-glutamine solution, 10 mg of bovine serum albumin (BSA) and 0.84 g of sodium citrate × 2H₂O per 1,000 ml; pH 7.4, 330 mosM) for 5 min at room temperature. Before use, the solution was oxygenated. A pulsating peristaltic pump (15 ml/min) was used. The
cortex (3–4 g) was removed immediately with a scalpel blade and pressed through a fine tissue press onto a 40-mesh nylon gauze. The tissue was then carefully stirred through the gauze without any pressure, using 100 ml of a 1:1 mixture of the described Earle’s solution without citrate and of the oxidized electrophoresis medium (11 mM triethanolamine, 11 mM acetic acid, 2.5 μM MgCl₂ × 6H₂O, 5 μM CaCl₂, 5 mM glucose, 285 mM sucrose, and 5 mg of BSA per 1,000 ml; pH 7.5 with 1 N KOH, osmolarity of 355 mosM, conductivity of 6.4 × 10⁻² μS/cm). The second kidney, which had been left in situ, was subsequently processed in the same way. The combined filtrates of the two kidneys were poured through a 20-mesh nylon gauze. The procedure was carried out at 4°C. The suspension was centrifuged for 5 min at 150 g; the pellet was resuspended in 40 ml of electrophoresis medium and centrifuged again. The resulting pellet was resuspended in 20 ml of medium; the suspension was slowly rotated (in a rotation evaporator) for 2 min in order to dissociate cell aggregates, and then filtered through a thin glass-wool layer packed into a Pasteur pipette. The glass wool did not absorb single cells but retained cell aggregates and tubule pieces. The filtrate was centrifuged at 150 g for 5 min; the pellet was resuspended in 1 ml of electrophoresis medium and centrifuged again. The resulting pellet was resuspended in 20 ml of medium; the suspension was slowly rotated (in a rotation evaporator) for 2 min in order to dissociate cell aggregates, and then filtered through a thin glass-wool layer packed into a Pasteur pipette. The glass wool did not absorb single cells but retained cell aggregates and tubule pieces. The filtrate was centrifuged at 150 g for 5 min; the pellet was resuspended in 1 ml of electrophoresis medium and injected into the electrophoresis apparatus. One milliliter of suspension contained about 40 × 10⁶ cells.

**Electrophoretic Separation**

The electrophoretic separation of the cells was carried out in an FFV apparatus (Bender & Hobein, München, Fed. Rep. of Germany) according to Hannig. The run was done at 230 mA, 132 V/cm, T = 5°C, buffer flow 2 ml/fraction/h, and sample injection 1 ml/h. 90 fractions were collected at 5°C. The collection tubes each contained 0.5 ml of the described Earle’s balanced salt solution without citrate but with the addition of 20 mg of BSA, 0.28 g of MgSO₄ × 7H₂O and 0.275 g of CaCl₂ per 1,000 ml. The fractions were used immediately for cell counting. In those experiments in which the separated cells were to be kept in culture, the FFV apparatus was made sterile with 3.5% formaldehyde solution, rinsed with sterile water, and then run with sterile electrophoresis medium.

**Electron Microscopy**

For thin sections, the cells were pelleted for 10 min at 200 g, fixed in 5% glutaraldehyde in electrophoresis medium, followed by Palade’s osmium tetroxide fixation, dehydration, and embedding in Epon. Sections were stained with uranyl acetate and lead citrate and were viewed at 60 kV in a JEM-100B microscope.

For scanning microscopy, the cells were fixed on mica for 15 min in 5% glutaraldehyde, followed by Palade’s osmium tetroxide fixation, and further processing in an exchange apparatus (24). Then they were dried in a critical point apparatus and sputtered with gold. Specimens were viewed at 15 kV in a JEM-35 microscope.

**Cultivation of Cells and Incorporation of [¹⁴C]Uridine**

Cells from the electrophoresis fractions were transferred into Dulbecco’s Modified Eagle Medium (MEM) supplemented with 2 mM glutamine, 2.2 g of sodium bicarbonate per 1,000 ml, 100 μg of streptomycin per milliliter, and 10% fetal calf serum. The cells (100,000 per milliliter) were kept in culture dishes at 37°C in a humid atmosphere (5% CO₂, 95% air).

To test incorporation of uridine, 0.5 μCi [¹⁴C]uridine (10–15 Ci/mmol) was added per milliliter of the cultures. At different times, 150-μl aliquots were precipitated in 5 ml of 5% trichloroacetic acid at 0°C; the precipitates were washed on Millipore filters (0.45 μm pore size, Millipore Corp., Bedford, Mass.), dried, and the activity was counted.

**Analytical Methods**

Renin (EC 3.4.99.19) was measured with a modified test of Haber et al. (13).

Eosin G dye exclusion was used in preference to trypan blue for routine examination of the isolated cells. Cell counts and cell volume determinations were performed in a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.).

**RESULTS AND DISCUSSION**

**Cell Preparation Procedure**

The technique mostly used to disintegrate tissue before making cell suspensions is the enzymatic treatment of the tissue with trypsin, collagenase, or collagenase and hyaluronidase. This procedure produces good yields of intact cells as is shown in many publications on liver and other tissues. In the present study, this technique was successfully used to prepare kidney cortex cell suspensions, but the subsequent separation of the cells into different cell populations by free-flow electrophoresis did not give satisfactory results, probably because of alterations of the cell surface produced by the enzymes used. For this reason, enzymes were not used in the present study. Jacob and Bhargava (21) have described a method for the preparation of liver cells by using Ca-binding ions. The idea is that junctional forces and complexes are weakened by the loss of Ca and that mild mechanical treatment then disintegrates the tissue. This procedure has also been used by Thimmapayya et al. (27). The procedure described in the present paper is a modification of this method. It produces a single-cell preparation which is suitable for cell separation in the FFV Apparatus. Debris, cell organelles, and membrane vesicles were re-
moved by three washes of the cells followed by centrifugation; tubule pieces, tissue elements, and cell aggregates were eliminated by filtration through nylon sieves and glass wool. The glass-wool layer did not absorb single cells specifically or unspecifically. Only cell aggregates were retained by this filtration. Fig. 1 shows a typical cell preparation. Most of the cells are single; few doubles and very few triples are present. No tubule pieces can be seen. All the cells excluded trypan blue dye, and about 90% of the cells were eosin G dye negative. Trypan blue, at least in the case of kidney cells, is not a reliable marker for the intactness of the cells. Even in very bad preparations which clearly contained injured cells, trypan blue was excluded from almost all of the cells.

It is known that Ca-deficient media injure cells. The respiratory properties are disturbed, and the morphology is altered (2). These effects were observed when EDTA was used as a Ca-binder in the perfusion of the kidneys. However, 5 min citrate proved to be tolerable. After the citrate perfusion, the Ca-concentration of the medium was immediately raised and the albumin concentration was increased.

Separation of the Cells by Free-Flow Electrophoresis

Fig. 2 shows a typical distribution of cells after an electrophoresis run. A main peak is visible with shoulders to the cathodic and anodic sides. Although in general the cell peaks after preparative free-flow electrophoresis do not appear to be sharp or well defined, a successful separation of the cells is normally achieved as shown below. After electrophoresis, fractions were immediately

FIGURE 1 Scanning electron microscopy of a cell suspension from rabbit kidney cortex obtained as described in Materials and Methods. Bar, 100 μm. × 140.
counted for cell numbers and tested with eosin G dye. The condition of the cells was not altered during electrophoresis, owing to the gentle procedure which uses fast separation times (the cells are in the electric field for only 3 min) and a separation medium which is basically identical to a culture medium. It seemed likely that cells with a large number of microvilli would be found in the slow-moving fractions. This effect has already been observed in rat kidney cell membranes (18).

Identification of Cells by Morphology

After the run, aliquots of all the cell-containing fractions were pooled and processed for electron microscopy. Fig. 3 shows a thin section through a preparation of the pooled cells. The cells appear to be intact. Microvilli, nuclei, mitochondria, the endoplasmic reticulum, and the cytoplasmic matrix are well preserved. Different cell types are

FIGURE 2 Cell count profile from a run of a kidney cortex cell suspension in an FFV apparatus. All the cells were deflected towards the anode. Renin activity is found only in fractions (Fract.) 16-21. Roman numerals indicate the different cell pools (see text).

FIGURE 3 Thin section through cells from an electrophoresis run. All the cell-containing fractions were pooled for preparing this specimen, in order to show the condition of the cells after electrophoresis. Bar, 5 μm. × 3,600.
The remainders of the electrophoresis fractions were pooled in groups as follows: pool I fractions 36–39, pool II fractions 29–31, pool III fractions 23–25, pool IV fractions 18–20, and pool V fractions 9–12. These pools were also characterized by electron microscopy. Pool I contains erythrocytes not completely removed by the perfusion. Fig. 4 shows a typical cell of the main fraction, pool II, possessing long microvilli. Fig. 6 shows clearly that the cells in this peak are homogeneous proximal tubule cells. Their diameter is 11–14 μm. Since, in the kidney cortex, proximal tubule cells are about four times more abundant than distal tubule cells (8), this high yield of proximal tubule cells is to be expected. The cells in pool III appear to be somewhat similar-looking cells but with much shorter microvilli (not illustrated) and are probably distal tubule cells. The cells in pool IV possess no, or only very short, microvilli. These cells contain large granules, 1.2–1.4 μm diameter, with a polar location within the cells (Fig. 5). The yield of these cells is very low (see Fig. 2).

**Identification of Cells by Biochemical Tests**

Since until now homogeneous cell populations from kidney cortex have not been available, proximal and distal tubule cells have not been characterized or identified biochemically. In the present study, proximal tubule cells were identified by their striking morphology. In the future, it may be possible to establish this identification by means of enzyme tests, since it is known from microdissection studies that different enzymes or enzymes, sensitive to different hormones, exist. (12, 20). In
particular, gluconeogenesis is thought to be a feature of proximal tubule cells, and phosphoenolpyruvate carboxykinase (EC 4.1.1.49) is a marker enzyme for this metabolic function. There is no reliable, well established biochemical marker for distal tubule cells.

The cells in pool IV, that is, the large granule-containing cells, were found to be renin-active. All the cell pools were tested for renin activity with a radioimmunoassay (13). Only the cells of pool IV showed renin activity. In addition, granules of the type seen in these cells have been isolated by density sedimentation and electrophoresis techniques and have been characterized as renin granules (see, for example, references 1, 6, 11, 19, 22, 23). Thus, the cells of pool IV are probably involved in the biosynthesis, the storage, and/or the metabolism of renin. The renin activity of pool IV was proven to be bound to the cells since free cell organelles and membrane fragments are not present in this fraction. The cells in pool V have not yet been identified. The yield in this fraction is very low. Attempts are being made to identify the cells with kallikrein (kininogenin) activity (EC 3.4.21.8) in the electrophoresis fractions.

**Viability of the Cells**

The vitality control of isolated cells is still controversial. A critical evaluation of this problem is given in reference 2. For routine use in the laboratory, dye exclusion, uridine incorporation, respiratory control, and electron microscopy are the available techniques. In the present paper, dye exclusion was used as a general control only, since it only shows severely injured cells. Besides electron microscopy, uridine incorporation into
trichloroacetic acid-precipitable protein proved to be an easy test to perform. The cells before and after electrophoresis, when kept in culture medium, incorporated uridine linearly over a period of about 8 h. Between 8 and 30 h, the uridine incorporation slowly reached a plateau. At that time, a pool of uridine not incorporated into the cells was still present in the medium. Since uridine incorporation is dependent upon several parameters (cell membrane, mitochondria, nucleus, and cytoplasm), it has not yet been possible to determine which cell function was destroyed or whether the medium is not optimal. It should be noted that the cells used for these studies came from adult animals and are highly specialized cells. Therefore, division of the cells was not expected. The time of 12 h of viability, however, is sufficient to allow stimulation and blocking experiments on the renin-active cells.

CONCLUSIONS
The present paper describes the electrophoretic isolation of proximal, distal tubule and renin-active cell populations from a single-cell suspension.
from rabbit kidney cortex. More certain identification of these cell populations might be possible by using the results of microdissection studies (12, 20). In addition, 1 g velocity sedimentation experiments are being performed to verify that the cell fractions are homogeneous. The viability of the cells from the electrophoresis run has to be studied in more detail, and the results described here should be supported by respiratory control experiments. However, the possibility of preparative separation of different cell types from a cell suspension which has been derived from a tissue is an achievement which should stimulate further experiments along this line. The use of homogeneous cell populations in transport studies (until now processes, or in analysis of drug action should give experiments on stimulation of metabolic proc-

We would like to thank Professor Hannig for his continuous interest in this work. We would also like to acknowledge the excellent technical assistance of Ms. G. Braun, Ms. U. Eichelbacher, and Ms. M. Löser.

Part of this paper was presented at the First International Congress on Cell Biology, September 5-10, 1976, Boston, Massachusetts.

Received for publication 22 February 1977, and in revised form 25 April 1977.

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