FUNCTION OF HORMONE-SECRETING CELLS IN CULTURE DURING CONTINUOUS PERFUSION WITH NUTRIENT MEDIUM

G. N. Pluzhnikova, V. P. Fedotov,
É. S. Yanakopulu, V. P. Lavrovskaya,
and É. I. Lezhnev

UDC 621.433+612.34].014.4.018-085.23

KEY WORDS: culture of adenohypophysis; culture of pancreatic β-cells; secretion of prolactin, ACTH, and insulin.

Research in recent years has shown that culture of various cells, including those secreting hormones, on different types of artificial porous membranes with a continuous flow of nutrient medium is a very promising procedure [1, 3, 6, 7]. All kinds of synthetic porous materials have been developed [4], but they are not all effective as regards adhesion and growth of cell cultures.

In previous papers a scheme of an apparatus with circulation of nutrient medium was presented. The apparatus was intended for culture of various cells, including primary cultures of hormone-secreting and hormone-sensitive cells on a porous support [1, 3]. Cells of the adenohypophysis and embryonic hepatocytes of rats were found to be capable of adhering, growing, and functioning under the specified conditions of circulation of the culture medium.

The aim of the present investigation was to study the dynamics of prolactin and ACTH secretion by cells of the adenohypophysis and also of insulin secretion by the pancreatic islet cells during long-term culture with circulation of the nutrient medium beneath the membrane on which the cells were placed. Another aim was to obtain confirmation that hormones secreted by the culture could be separated, depending on the size and properties of their molecules, by using artificial membranes with different pore diameters.

EXPERIMENTAL METHOD

A suspension of adenohypophysis cells was obtained by the method in [2]. Medium 199 with 2% bovine serum was used. Pancreatic β-cells were obtained by the method used in the Laboratory of Biological Standardization of Hormones [5]. Cells were isolated from the pancreas of newborn rats by means of 0.01% collagenase solution in 0.25% trypsin solution. The cells were cultured in medium 199 with 3.5% bovine serum. The medium contained a normal concentration of glucose (5.5 mM). Experiments were carried out on the two types of cultures on the 7th day of cultivation.

To grow adenohypophysis cells, cellulose acetate membranes of UAM-100 and UAM-200 types (pore diameter 5-10 and 15-20 nm respectively) were used. The membranes were manufactured by the Experimental Factory of the All-Union Research Institute of Synthetic Resins. Adenohypophysis cells also were cultured on a membrane of leached sodium borosilicate glass with an effective pore radius of 5 nm. The glass was manufactured at the Glass Research Institute. Pancreatic β-cells were grown on a membrane of sodium borosilicate glass with a pore radius of 5 nm.

The cultivation chamber of the apparatus was divided by one of the porous membranes into two compartments. Cells were placed in 5 ml medium in the upper compartment, whereas the lower compartment was connected to the circulating system. The cells thus were in com-

Laboratory of Biological Standardization of Hormones, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Processes and Apparatus of Cell Culture, Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 9, pp. 12-14, September, 1982. Original article submitted January 27, 1982.

 0007-4888/82/9409-1174$07.50

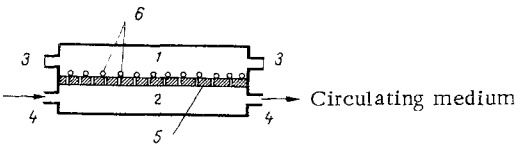

Fig. 1. Diagram of cultivation chamber: 1) top compartment, 2) bottom compartment of chamber; 3) top connecting pipes, 4) bottom connecting pipes, 5) porous membrane, 6) cells on surface of membrane.

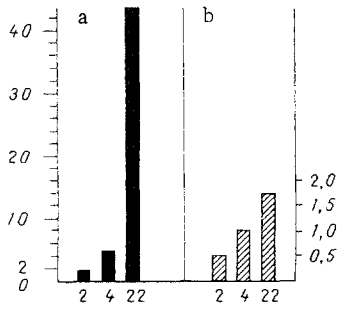

Fig. 2

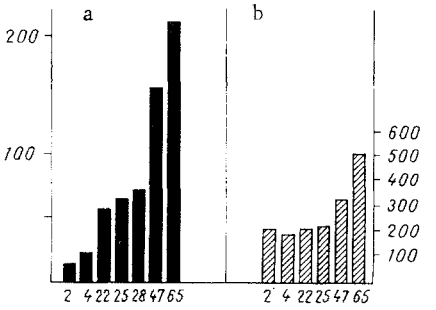

Fig. 3

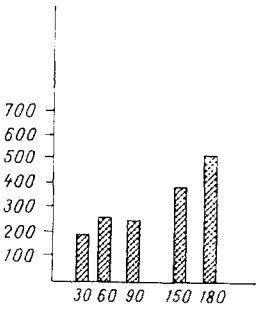

Fig. 4

Fig. 2. Secretion of prolactin and ACTH into medium during culture of adenohypophysis cells on sodium borosilicate glass (effective pore radius 5 nm): a) prolactin in top compartment of chamber (no prolactin was found in the circulating medium), b) ACTH in circulating medium. Abscissa, incubation time (in h); ordinate: left — prolactin concentration (in μg/ml), right — ACTH concentration (in ng/ml).

Fig. 3. Secretion of prolactin and ACTH into circulating medium during culture of adenohypophysis cells on membrane of UAM-200 type (pore diameter 15-20 nm): a) prolactin in circulating medium, b) ACTH in circulating medium. Remainder of legend as to Fig. 2.

Fig. 4. Secretion of insulin into circulating medium during culture of pancreatic β-cells on sodium borosilicate glass (effective pore radius 5 nm). Abscissa, incubation time (in min); ordinate, insulin concentration in circulating medium (in microunits/ml).

munication with the circulating medium only through pores in the membrane (Fig. 1). Before the experiment began, i.e., until the 7th day of culture, the chamber containing the cells was kept separately from the apparatus in an incubator. After a routine change of medium the chamber was connected to the system, filled with nutrient medium in a volume of 20 ml (into the compartment beneath the membrane), and the fluid was set in motion at a constant flow rate of 60 ml/h by means of a peristalic pump. The other parameters of the automatic control system also were kept constant in the course of the experiment: pH of the medium 7.2-7.4, temperature 37°C.

Samples for determination of hormone concentrations in the medium, measuring 0.2 ml in volume, were taken with a sterile syringe through the upper or lower connecting pipes of the chamber, not earlier than 30 min after the last change of medium and restarting the flow of fluid.

The prolactin concentration in the medium was determined by a radioimmunologic method, using rabbit antibodies against rat prolactin. The prolactin and antibodies against it were obtained by V. V. Abramova, of the Laboratory of Biological Standardization of Hormones. The concentrations of ACTH and insulin in the corresponding culture medium were determined radioimmunologically with the aid of commercial kits from CEA-Sorin (France).

EXPERIMENTAL RESULTS

The concentrations of prolactin and ACTH in the culture medium of the adenohypophysis and also of insulin in the culture of β-cells with continuous circulation of nutrient medium

increased in the course of a long period of incubation (Figs. 2-4). This indicates that cells in culture under continuous medium circulation conditions are viable and possess secretory activity. Three series of experiments were carried out on the culture of pituitary cells, using three types of membranes. In the experiments of series I the cells were seeded on sodium borosilicate glass. Incubation in a continuous-flow system continued for 22 h. The results showed that prolactin does not pass through pores with an effective radius of 5 nm, but remains and accumulates in the top compartment of the chamber; ACTH, on the other hand, passed freely into the circulating medium (Fig. 2).

When UAM-100 film with a pore diameter of 5-10 nm was used prolactin also remained in the upper compartment of the chamber, whereas ACTH, as in the first case, was found in the circulating medium. Incubation continued for 17 h. When adenohypophysis cells were cultured on UAM-200 membrane with a pore diameter of 15-20 nm, both prolactin and ACTH passed through into the circulating medium. Hormone concentrations were determined in this experiment for 65 h (Fig. 3).

Pancreatic β-cells were grown on sodium borosilicate glass with an effective pore radius of 5 nm. The insulin concentration was determined every 30 min for 3 h. Insulin was found to pass freely through the pores of the membrane into the circulating medium and its concentration increased with time (Fig. 4).

The experiments showed that, of the membranes used, the most suitable is film of the UAM type. This material is transparent and is easily sterilized (2 h in 30° ethanol). Because the membrane is transparent, the morphological state of the cells during growth can be inspected. The cells also adhered and grew well on sodium borosilicate glass, but its complex microstructure made microscopic examination of the cells difficult.

It can be concluded from the results that culture of cells of endocrine organs on porous material with continuous perfusion of medium beneath the membrane possesses definite prospects. The use of porous membranes enables the liberation of a particular hormone into the medium to be studied selectively. Moreover, after certain improvements have been made in the future to the circulating system, it will evidently be possible to culture hormone-secreting and hormone-sensitive cells in closed and open systems of circulation of the medium. In that way different models of humoral interaction between specialized cells will be feasible.

LITERATURE CITED

1. B. K. Gavrilyuk, V. P. Lavrovskaya, É. I. Lezhnev, et al., Tsitologiya, No. 2, 226 (1981).
2. I. S. Komolov, L. G. Morozova, and I. Fazekas, Byull. Éksp. Biol. Med., No. 2, 215 (1978).
3. V. P. Lavrovskaya, É. I. Lezhnev, A. I. Lelikova, et al., Tsitologiya, No. 2, 233 (1981).
4. Membrane Technology: a New Trend in Science and Technology [in Russian], Vladimir (1977).
5. V. P. Fedotov, I. S. Komolov, and N. V. Sadovnikova, Probl. Éndokrinol., No. 4, 57 (1978).
6. R. Knazek, P. Cullino, and W. Kidwell, U.S. Patent 3821087 (1974).
7. P. Shratter, Methods Cell Biol., 14, 95 (1976).