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Effect of Dopamine on Viability of BHK-21 Cells

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We studied the effects of dopamine added to culture medium on survival of floating or adherent BHK-21 cells differing by organization of actin cytoskeleton. The viability of floating cells more drastically decreased with increasing dopamine concentration and duration of exposure than that of adherent cells. The cells worse adhered to the substrate and formed a monolayer. The formed monolayer degrades, cell borders become blurred, cells, polygonal in the control, are rounded. Preliminary blockade of dopamine receptors with haloperidol, inessential for cell survival and morphology, does not prevent the destructive effect of dopamine on the cells. Ultrastructural study revealed increased density of filamentous actin threads in deep compartments of cell cytoplasm after dopamine treatment, this increase being more pronounced in cells grown in suspension. Bearing in mind the polymerizing effect of dopamine on globular actin *in vitro* and the fact that the content of this protein in floating cells is higher than in adherent cells, we can conclude that the decrease in viability of BHK-21 cells is caused by interaction of dopamine with cytoplasmic globular actin.

Key Words: *BHK-21 cells; dopamine; haloperidol; survival; morphology*

It was hypothesized that, apart from cell-activating ligand interactions with the corresponding receptors on the membrane, neurotransmitters and hormones can nonspecifically modulate (as chemical substances) cell membrane and cytoplasmic proteins [4,8,10]. Model experiments confirmed this hypothesis, at least for biogenic amine dopamine (DA). It was shown *in vitro* that DA interacts with isolated monomeric actin (the most prevalent protein of any eukaryotic cell) and polymerizes it [1,4]. Moreover, DA penetrating through the membrane causes polymerization of monomeric actin in phospholipid vesicles (liposomes) [5]. It is not yet possible to prove for living cells that membranes and cytoplasmic proteins, specifically actin, can serve as DA targets. The available morphological facts of DA-induced stabilization of cortical actin cytoskeleton in primary culture of rat pituitary lactotropic (prolactin-secreting) cells and hypertrophy of actin desmosome-like synaptic contacts in goldfish Mauthner neurons might be interpreted as an evidence of the direct effect of DA on cytoplasmic actin [4,13]. However, DA can affect the morphology of these cells not directly, but via multi-level signal pathways. The role of dopamine receptors in the organization of actin cytoskeleton remains little studied; it is unclear whether DA penetrates through the plasma membrane from the extracellular medium into the cytoplasm of living cells. Many problems impeding *in vivo* studies of DA interactions with cells can presumably be solved by using cells whose function is not directly regulated by DA, for example, cultured BHK-21 fibroblast-like cells, previously used for evaluation of proliferative activity

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under conditions of hypoxia [2]. Similarly as normal fibroblasts, they are actin-rich cells. Filamentous actin is mainly located in the microvilli, cortical layer, cell-cell contacts, and sites of cell adhesion to the substrate. BHK-21 cells attracted our attention because they exist in two phenotypical variants in suspension and in monolayer due to different organization of the actin cytoskeleton. Suspended cells are always round and have virtually no actin, except a thin perimembrane cortical layer. The main pool of cytoplasmic actin is presented by globular (not polymeric) form [12], which we regard as the potential substrate for direct interactions with DA. Adherent rhomboid or polygonal cells have a well-developed cytoskeleton: a strong cortical layer, stress-fibrils, and cell-cell and focal contacts. It is formed from filamentous (polymeric) actin not reacting with DA. Different proportions of globular and filamentous actin in BHK-21 cells in suspension and monolayer create a prerequisite for adequate evaluation of the involvement of the actin cytoskeleton in nonspecific interactions of DA with living cells.

We studied the effects of DA on viability of BHK-21 cells, including those under conditions of dopaminergic receptor blockade with classical drug haloperidol (HP).

MATERIALS AND METHODS

Baby hamster kidney fibroblasts (BHK-21) from the Russian Collection of Cell Cultures, Institute of Cytology, were used in the study. The cells were cultured in Carrel flasks (25 cm^2/5 ml) or Petri dishes (12 cm^2/2 ml) at 37°C and 5% CO_2 in RPMI-1640:DMEM (1:1) with 10% bovine serum and gentamicin (80 μg/ml). The cells were inoculated in doses of 400,000 and 100,000 cells on the surface in flasks and dishes, respectively.

In order to rule out the side effect of DA oxidation, antioxidant (sodium metabisulfite; 200 μM) was added to culture medium [11]. The final concentrations of DA were 10^{-5}, 10^{-4}, and 10^{-3} M. DA was added to cell suspension simultaneously with inoculation (before cell flattening) or 6 h after inoculation, when the cells adhered and started to flatten. Cells without DA with sodium metabisulfite in the same concentrations as DA served as the control; intact control were cells without DA and sodium metabisulfite. Cell survival was evaluated by the formula:

$$\text{Survival } (\%)=N_2\times 100(N_1),$$

where N_1 is the number of inoculated cells, and N_2 is cell count on days 1, 2, and 3 in the control and in the presence of DA.

Suspended cells were counted in a Goryaev chamber on days 1, 2, and 3 after addition of DA by staining with 0.5% trypan blue solution. In cases when DA was added to culture medium after cell adhesion to the substrate, the survival was evaluated by morphological parameters (status of individual cells in the center and at the periphery of colonies and by monolayer density in several randomly selected fields of view.

In preliminary studies [6] we found that HP in a dose of 10^{-5} M completely blocked all cell receptors without causing cell death. Haloperidol was added to culture medium directly before cell inoculation.

The preparations were processed by methods used at our laboratory for preparations for electron microscopy, so that the same cells were studied in succession at histological and ultrastructural levels [3]. The cells were fixed and embedded in epon in the same flasks in which they had been cultured and examined under a stationary (ground-type) NU-2E light microscope (Carl Zeiss) fitted with a digital Nikon Coolpix 995 camera and under a Tesla BS-500 electron microscope.

RESULTS

Evaluation of BHK-21 cell culture viability revealed a cytotoxic effect of DA manifesting in reduction of cell viability during culturing. This effect was clearly seen in suspended cells cultured with DA added to the culture medium before cell adhesion to the substrate (Fig. 1). The count of control BHK-21 cells increased progressively (almost 4-fold after 3 days). The presence of DA in a concentration of 10^{-5} M in culture medium little affected cell status; control and experimental preparations virtually did not differ. Increasing DA concentration to 10^{-4} M caused gradual death of cells, the percentage of living cells in comparison with the initial number of inoculated cells being 94, 65, and

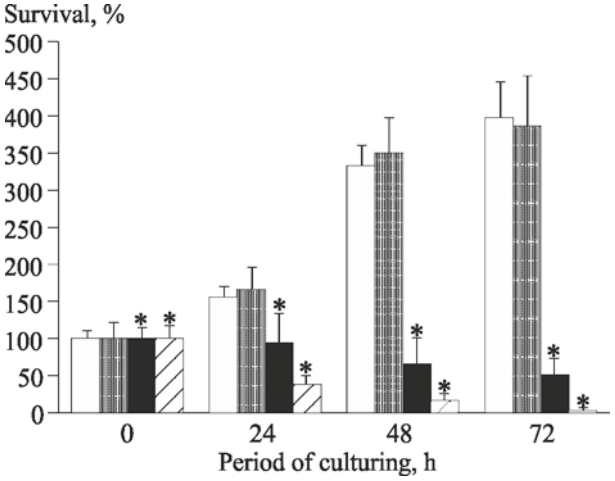

Fig. 1. Effect of DA on survival of BHK-21 cells in suspension after 24, 48, and 72 h of culturing. Light bars: control; vertically hatched bars: DA, 10^{-5} M; dark bars: DA, 10^{-4} M; obliquely hatched bars: DA, 10^{-3} M. *p<0.05 compared to the control.

51% on days 1, 2, and 3, respectively. Incubation of cells with DA in a concentration of 10^{-3} M caused a more pronounced negative effect. Cell survival after 24 and 48 h was 38 and 16%, respectively, and cell death was observed on day 3. Increase in DA concentration and prolongation of exposure did not modify round shape of cells, but caused at first their adhesion, then fusion with the formation of amorphous clots, and then destruction (Fig. 2, *a-d*).

Experiments showed that first only solitary cells from the suspension adhered to the substrate without dividing and flattening. Stationary formation of islets of flattened cells with subsequent formation of the monolayer started only at the end of the first 24 h of culturing. Hence, only starting from this period we could form an adequate conclusion on monolayer confluence. In the control, the density of the forming monolayer of polygonal cells was 80-85% on day 2 and 100% on day 3 (Fig. 2, *e*, *i*). Addition of DA in a concentration of 10^{-5} did not much affect cell morphology. On days 2 and 3, the monolayer reached 70 and 100% confluence (similarly as in the control; Fig. 2, *f*, *j*). Incubation of the monolayer with DA in a concentration of 10^{-4} M led to greater reduction of monolayer density, which was about 50% after 48 h (Fig. 2, *g*) and 25-30% on day 3 (Fig. 2, *k*), which attested to progressive cell death. Degradation of the monolayer and its disintegration into small islets consisting, nonetheless, from normally looking cells, were seen in the visual field (Fig. 2, *k*). All adherent cells

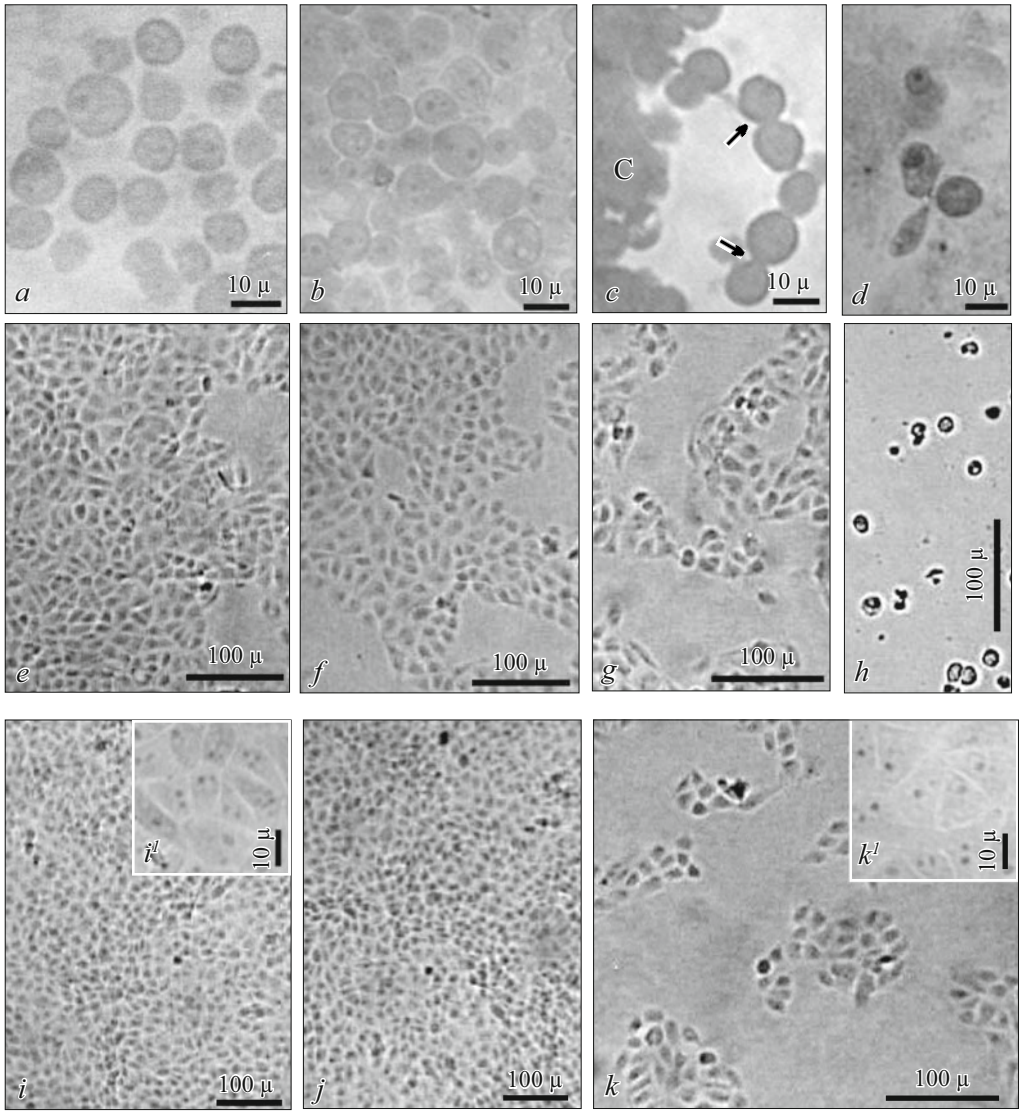

Fig. 2. Morphology of BHK-21 cells after DA treatment. *a-d*) DA added to cells before adhesion to substrate. *a*) control; *b*) DA, 10^{-5} M; *c*) DA, 10^{-4} M. Paired adhesion of cells (arrows) and formation of amorphous clot with blurred interface (C); *d*) DA, 10^{-3} M. *e-k*) DA affects on cells adhering to substrate for 48 h (*e-h*) and 72 h (*i-k*). *e*, *i*) control (i^1: at greater magnification); *f*, *j*: DA, 10^{-5} M; *g*, *k*: DA, 10^{-4} M (k^1: at greater magnification); *h*) DA, 10^{-3} M.

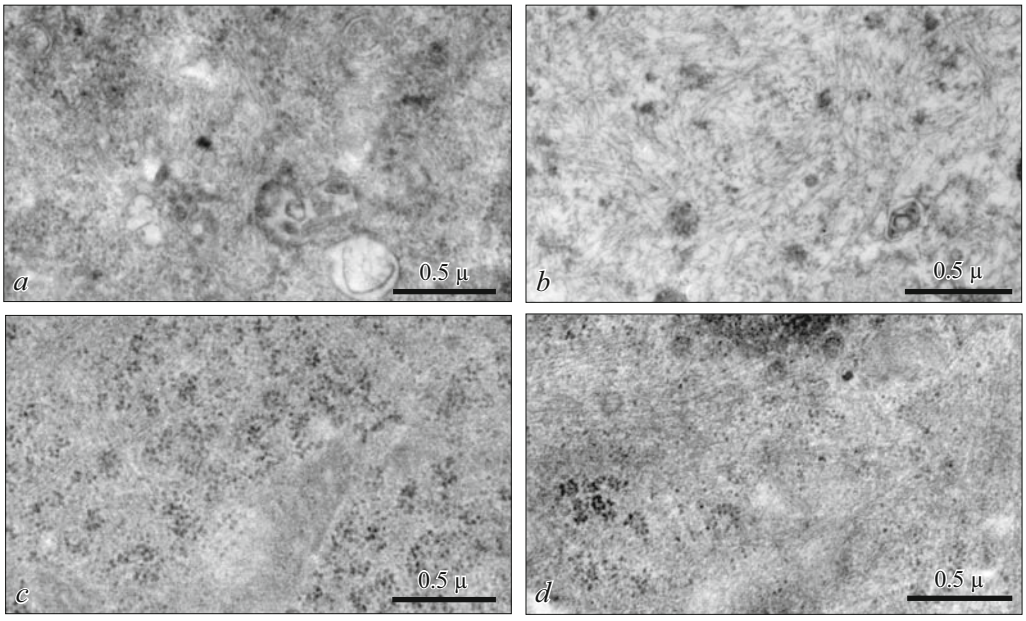

Fig. 3. Morphology of BHK-21 cells after DA treatment. *a-d*) ultrastructure of BHK-21 cells after DA treatment. *a*, *b*) DA effect on suspended cells; *c*, *d*) DA effect on adherent cells. *a*, *c*: control. *b*, *d*: cells incubated with 10^{-3} and 10^{-4} M DA, respectively.

cultured with 10^{-3} M DA were round and much damaged. On day 2, the confluence was 20-25% and on day 3 it was just 0-10%, in other words, no monolayer was in fact forming (Fig. 2, *h*).

The study of HP effect on adherent BHK-21 cells showed no effect of D2- and α-adrenergic receptor blockade on cell morphology. On the other hand, combined treatment with DA and HP did not cancel the negative impact of DA for BHK-21 cell viability and morphology (data not shown).

The ultrastructure of suspended and flattened BHK-21 cells in the control and after long treatment with DA is presented in Fig. 3. On day 3 of culturing, deep cytoplasm of suspended cells in the control contained virtually no actin filaments (Fig. 3, *a*), while treatment with DA in a concentration of 10^{-3} M led to the formation of a dense network of chaotic filaments (Fig. 3, *b*). On day 3, actin filaments, orderly and moderately developed in the control monolayer cells (Fig. 3, *c*), after 10^{-4} M DA treatment were hypertrophic and disorderly (Fig. 3, *d*).

Hence, BHK-21 cells were sensitive to the cytotoxic effect of DA, their sensitivity directly correlated with the content of actin in the globular (not polymerized) aggregate state, which was higher in the cytoplasm of free suspended cells than in the cytoplasm of flattened cells with high content of filamentous (polymerized) actin [12]. According to the results of model studies, globular actin serves as the target and substrate for DA-induced direct formation of filaments [1,4,5]. Hence, the effects of DA on BHK-21 cell viability and morphology, observed in our experiments, can be explained by the direct polymerizing effect of DA on cytoplasmic globular actin. Previously detected changes in cell shape and hypertrophy of the cell cortical layer in lactotropes and desmosome-like contacts of Mauthner neurons [1,13] can be interpreted from this standpoint. Ultrastructural data on DA-induced hypertrophy of filamentous actin network in BHK-21 cell cytoplasm (more detailed data in [6]) and recent cytochemical data on DA penetration into BHK-21 cells and its integration in the actin filaments [7] confirm the possibility of non-ligand (trophic) DA interactions with living cells, as was assumed previously. Ultrastructural data on filamentous actin threads hypertrophy in Vero cell cytoplasm under the effect of jasplakinolide [9] (a toxin penetrating through the membrane and stabilizing actin) were recently obtained. The similarity of the effects of this toxin and DA on living cells suggests regarding DA as a special agent, penetrating into the cell and polymerizing actin. It is noteworthy that BHK-21 cells are transformed, they can be regarded as a tumor cell model. Hence, these data suggest the use of these cells as the object for studies of carcinogenesis mechanisms and drug therapy for cancer.

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