INHIBITION BY COLCHICINE OF IMMUNOREACTIVE VASOACTIVE INTESTINAL POLYPEPTIDE RELEASE FROM ANTERIOR PITUITARY CELLS IN CULTURE

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ABSTRACT

We investigated the effect of colchicine on the release of vasoactive intestinal polypeptide (VIP) from adenohypophyseal cells in culture, measuring VIP by radioimmunoassay. Additionally, prolactin (PRL) and growth hormone (GH) release was studied. Exposure of cultured pituitary cells to colchicine for 3 or 6 h caused a significant decrease in the release of immunoreactive VIP (IR-VIP) into the media, concomitantly with an increase in IR-VIP in cells. Long-term exposure to the drug (12 and 24 h) leads to a dramatic decrease in IR-VIP in cells. These findings are similar to those obtained for PRL and GH. Though the exact nature of VIP-producing adenohypophyseal cell(s) is not known, the results suggest that microtubules play an important role in the mechanism responsible for VIP release from adenohypophyseal cells in culture.

Vasoactive intestinal polypeptide (VIP), originally isolated from porcine duodenum (30), has been shown to be present in the anterior pituitary by means of various experimental methods (2, 3, 10, 19, 31, 36).

Although, it seemed initially that VIP and prolactin (PRL) are produced in the same cell (17), a different population of VIP-producing cells in the anterior pituitary, functioning as regulator of lactotrophs, have recently been described (29, 36).

It is well-known that colchicine, a mitosis-inhibiting drug, interferes with the secretory activity of various endocrine and exocrine cells (7, 8, 12, 15, 26-28, 37). This drug depolymerizes microtubules to tubulin and suppresses the transport of granules, resulting in an accumulation of secretory products. Thus colchicine is commonly used to increase the reaction intensity in various immunocytochemical studies (6, 9, 16, 20-25, 32-34, 38).

In order to know whether microtubules are involved in the secretion of different adenohypophyseal hormones, the effect of colchicine on anterior pituitary cells in monolayer culture has pre-

viously been investigated (1, 11). However, the effect of this drug on the release of adenohypophyseal VIP has not yet been investigated.

We attempted in the present study to elucidate the effect of colchicine on the VIP release from anterior pituitary cells in culture. For comparison, we have also examined the well-known release of PRL and growth hormone (GH).

MATERIALS AND METHODS

Cell Culture

Male Wistar rats (180-200 g) were sacrificed by decapitation. The anterior pituitary glands were removed, collected in Earle's balanced salt solution (EBSS) and rinsed three times with the same solution. The pituitaries were then placed in a 15-ml tube containing 0.15% MgCl₂, 0.1% papain, 0.1% deoxyribonuclease, and 0.1% neutral protease and dispersed. The pituitary fragments were incubated in a water-jacketed incubator for 1 h at 37°C. The rate of dispersion was increased by gently drawing the fragments in and out of a Pasteur pipette every

30 min. After 1 h the cell suspension was centrifuged at 3,000 rpm for 5 min. The supernatant was discarded, and the sedimented cells were resuspended in an appropriate volume of Dulbecco's modified Eagle's medium (DMEM) containing 3% fetal serum, 100 U/ml penicillin, 100 U/ml streptomycin, and 0.1 mU glutamine was freshly added (4, 35).

Cells were placed in tissue culture dishes (30 × 15 mm) at a concentration of 5×10^5 cells/1.5 ml/plate, and placed in an incubator at 37°C under an atmosphere of 5% CO₂/95% air. Media were changed on the third day of incubation. Experiments were performed after 4 days of incubation.

Experiments with Colchicine

At the beginning of each experiment, the media were discarded, and the cells were rinsed with EBSS; and fresh DMEM without serum (1.5 ml) and without or with colchicine (3 µg/1.5 ml medium) was then added. After 3, 6, 12 or 24 h, the culture dishes were chilled on ice, and the media were collected. Cells were washed with ice-cold 0.01 M phosphate/0.15 M NaCl (pH 7.4) buffer (PBS), resuspended in 1 ml of PBS and sonicated for 15 sec. Media and cell extracts were centrifuged at 3,000 rpm for 30 min. Supernatants were kept frozen for later measurement of immunoreactive VIP, GH and PRL. Protein concentrations were measured according to Lowry *et al.* (14), and were found to be similar in each of the dishes.

Immunoassays

PRL and GH were assayed, with modifications, according to the protocol provided by the National Pituitary Hormone Distribution Program (NIAMMD, Bethesda, MD), by a double antibody radioimmunoassay (PRL or VIP), or by protein A radioimmunoassay (GH). VIP was assayed as previously described (14).

Statistical Analysis

This was made with the Student *t*-test and with the ANOVA test. Values at $P < 0.05$ were considered statistically significant.

RESULTS

Effects of Colchicine on VIP Release

Incubation of pituitary cells with colchicine signifi-

Fig. 1 IR-VIP in media (a), cells (b) and its total amount (c) in the control and colchicine-treated culture dishes. Values are expressed in picograms/plate ± SD. * $P < 0.05$, and ** $P < 0.01$ vs control. Open column, control (10 dishes); striped column, colchicine-treated (10 dishes)

cantly decreased IR-VIP release into the media at all times studied (3, 6, 12 and 24 h), whereas the amount of immunoreactive VIP (IR-VIP) in cells was increased markedly after 3 h, nearly equivalent with the control at 6 h, and decreased dramatically at 12 and 24 h (Fig. 1, a and b). In the control, basal IR-VIP release was increased during incubation, being maximum at 24 h, whereas IR-VIP in cells remained almost unchanged (Fig. 1, a and b). The

Fig. 2 IR-PRL in media (a), cells (b) and its total amount (c) in the control and colchicine-treated culture dishes. Values are expressed in micrograms/plate ± SD. * $P < 0.05$, and ** $P < 0.01$ vs control. Open column, control (10 dishes); striped column, colchicine-treated (10 dishes)

total IR-VIP showed no clear differences between the control and colchicine-treated-dishes at 3 and 6 h (Fig. 1c). However, a long-term exposure to colchicine (12 and 24 h) caused a significant decrease in the total amount of IR-VIP ($P < 0.01$) compared with the control (Fig. 1c).

The total IR-VIP in the control dishes was slightly increased throughout the incubation time, indicating the viability of VIP-producing cells

Fig. 3 IR-GH in media (a), cells (b) and its total amount (c) in the control and colchicine-treated culture dishes. Values are expressed in micrograms/plate ± SD. * $P < 0.05$, and ** $P < 0.01$ vs control. Open column, control (10 dishes); striped column, colchicine-treated (10 dishes)

under the present culture conditions (Fig. 1c).

Effects of Colchicine on PRL or GH Release

Effects of colchicine on the release of PRL or GH were, in general, similar to those observed for VIP. Treatment with colchicine caused a clear decrease in IR-PRL or IR-GH in media ($P < 0.01$) (Figs. 2a and 3a). Their intracellular content was increased

at 3 and 6 h (Figs. 2b and 3b). Long-term incubation (12 or 24 h) resulted in a considerable decrease in their intracellular content, amounting to 50% or higher.

The total amount of IR-PRL or IR-GH did not statistically differ ($P>0.05$) at 3 and 6 h between the control and colchicine-treated cells (Figs. 2c and 3c). A long-term exposure to colchicine resulted in a marked decrease in both hormones when compared with the control ($P<0.01$) (Figs. 2c and 3c).

DISCUSSION

It is well known that VIP is synthesized in adenohypophyseal cells (2, 3, 19, 31, 36). Our present results indicate that adenohypophyseal cells in culture can produce and release VIP. It is also demonstrated that colchicine inhibits the release of not only PRL and GH, but also the release of VIP from cultured cells.

Colchicine rapidly inhibits the VIP release (3 h), with an increase in the intracellular peptide content. Similar effects can be observed at 6 h. However, in a long-term exposure to colchicine the intracellular IR-VIP content was dramatically decreased, suggesting either an increase in VIP degradation or a diminished VIP-synthesis. Colchicine exerts similar effects on GH or PRL.

With regard to PRL, our present results agreed with findings in a previous paper (1). The paper described a rapid inhibition by colchicine of PRL release (4 h) in anterior pituitary cells in monolayer culture; this inhibition was accompanied by an increase in the intracellular content even after the 24-h treatment. A drastic decrease in the amount of intracellular PRL occurred after this period. We observed a marked decrease in intracellular decrease PRL at 12 h, but this minor discrepancy may be explained in terms of the different dose of colchicine used.

Labrie *et al.* (11) showed a time-dependent inhibitory effect of vincristine on the GH release and on the basal and stimulated release of PRL in cultures of hemipituitaries (1 h after incubation). Following a short-term incubation (4 h) with colchicine or vincristine, disappearance of microtubules accompanied by an accumulation of secretory granules were seen, whereas a long-term exposure resulted in a marked decrease in the number of granules and the appearance phagocytic features (1, 11). These morphological findings were found in all

pituitary cell types.

Although the nature of the pituitary VIP-producing cells has not been identified yet, similar changes in microtubules are expected to occur in these cells. This implies that microtubules are involved in the secretion processes in the VIP-producing cells. The exact knowledge of VIP-producing cells will clarify whether VIP exerts a paracrine (5) rather than an autocrine effect (18).

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