Inhibition by Cortisol of the Favourable Action of Lysine-Vasopressin on the Growth of HeLa Cell Cultures

The effects of posterior pituitary hormones on cells in tissue culture have not as yet been thoroughly studied. A previous work done with PIETTE and CHALAS[1] described the action of lysine-vasopressin (LVP) added to the HeLa cell culture medium; this paper reports the effect of simultaneous addition of LVP and cortisol (compound F).

Method. Using PAUL's technique[2] of stationary cultures in test-tubes, we placed 2–3.5 × 10^6 cells into 70 ml of the Pasteur Institute's semi-synthetic culture medium which contains a protein hydrolysate in balanced salt solution, plus 10% of foal serum. After 24 h of incubation (T_0) at 37 °C, some tubes were trypsinized for measurements of nitrogen content and cell counts. Into the other tubes was introduced the new medium containing the experimental hormones. This medium was renewed 2 days later. The following day (fifth day), the remaining tubes were trypsinized and the same measurements carried out. Nitrogen determinations were done by the LOWRY[3] method. Effects of the following procedures were studied: (1) addition of 25 μU/ml of synthetic LVP (Sandoz); (2) addition of 25 μU/ml of LVP plus 1 μg/ml of cortisol (lyophilized hydrocortisone ROUSSEL); (3) cortisol alone at the same dose of 1 μg/ml. The control medium contained solvents (heat-inactivated hormone).

Results. In the first series of experiments (Table I), the effects of LVP alone and LVP plus cortisol were compared. The growth of former group (LVP alone) was significantly more rapid. The quantity of nitrogen per tube was 27.71 ± 0.83 μg/tube compared to 25.15 ± 0.67/tube for the LVP + F group. The number of cells was also greater, 234.1 ± 15.9 × 10^3 tube for the LVP group *v.* 176.5 ± 16.0 × 10^3/tube for the group LVP + F.

In the second experimental series, the effects of cortisol alone were compared with those of a control solution. No significant differences were observed.

The results are expressed in the Figure as the % increase over the initial value for the various experimental procedures.

Discussion. LVP causes an increase in the rate of the growth of HeLa cell cultures. These results are in agreement with those of a previous experiment[1]: with the same dose of 25 μg/ml, we have observed an increase of 294% in the proteins and of 264% in the cell number of cultures treated with LVP, as compared to an increase of 163 and 142% respectively, in controls. Cortisol itself has well-known effects: at a dose of 50 μg/ml it causes after 8 days of incubation a decrease of cell multiplication and an increase of total proteins[4]. With smaller doses, similar to that which we have chosen, the effect of cortisol is apparently variable[5]. In our experimental conditions, cortisol alone (1 μg/ml) has no significant effect. On the other hand, if it is added together with LVP, it decreases signficantly the favourable effect which LVP has on cell multiplication. The mechanism of this action cannot be fully explained with the information provided by our experiments. One can suppose, following AUJARD and CHANY's hypothesis, that cortisol causes an increase in the rate of DNA synthesis, therefore LVP would have to act at another cytophysiologic point, for instance the duration of the DNA postsynthetic phase. It should also be pointed out that with cortisol + LVP the cell contains more protein than with LVP alone (nitrogen/cell number = 0.14 for cortisol + LVP and 0.12 for LVP alone). There is, of course, a possibility that each hormone acts differently upon mitotic process and cell growth[6].

Table I. Comparison of the actions of LVP (25 μU/ml) and LVP (same dose) plus cortisol (F) (1 μg/ml) on the growth rate of cell cultures

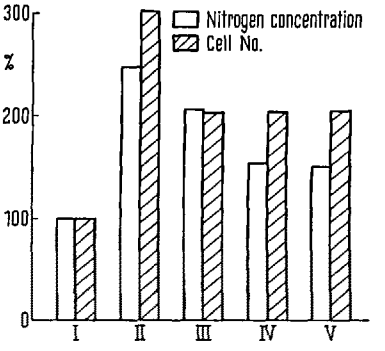
	T_0 (35)	LVP (35)	LVP + F(35)
Nitrogen (μg)/tube	7.98 ± 0.72	27.71 ± 0.83	25.15 ± 0.67
Cells (× 10^3)/tube	57.3 ± 3.7	234.1 ± 15.9	176.5 ± 16.0

T_0, initial values. No. of analyses in brackets. Data of LVP column are significantly higher than LVP + F column ($P < 0.02$ for nitrogen and cells).

Table II. Action of F (1 μg/ml) on the cell culture growth rate

	T_0 (35)	F (35)	Controls (35)
Nitrogen (μg)/tube	11.88 ± 0.94	32.19 ± 1.64	32.09 ± 2.41
Cells (× 10^3)/tube	79.2 ± 3.9	245.9 ± 18.4	247.7 ± 19.8

Data of F column and controls are not significantly different.



Percentage increases of nitrogen concentration and of cell number calculated from the Tables. Results are related to a same standard value. I controls (initial values). II LVP. III LVP + F. IV F. V controls (final values).

Résumé. L'action stimulante de 25 μU/ml de lysine-vasopressine de synthèse sur la croissance des cultures de cellules HeLa est inhibée par l'addition de 1 μg/ml de cortisol.

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