

EFFECT OF PROGESTERONE ON THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE 2 IN VITRO

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Summary: This project involved an investigation into the effect of progesterone on the replication of Herpes simplex virus Type 2 in a Vero-cell culture.

The first part of the experiment relates to preincubation of the cells with progesterone in the duration of 48 hours. After inoculation, replication of the virus was continued in the presence of progesterone. Samples for titration were taken 10, 21 and 32 hours after inoculation for the purpose of determining the single-cycle replication curve of Herpes simplex virus Type 2 in the presence of this hormone. An identical procedure was followed in the control part of the experiment, though without using progesterone.

The results thus obtained show that there is no significant difference in the production of infectious particles of Herpes simplex virus Type 2 in one cycle on making a comparison between different experimental conditions (presence and absence of progesterone).

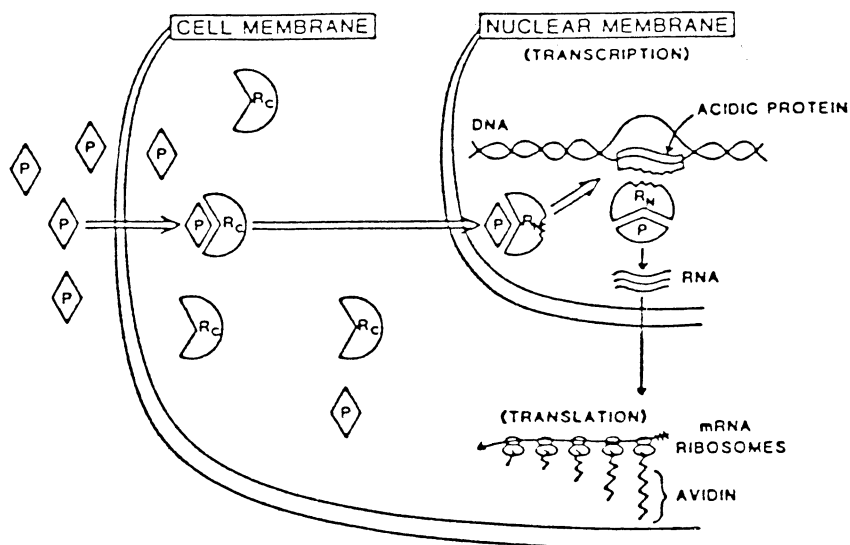
The incidence of genital infections caused by the Herpes simplex virus (HSV) has been growing continuously in recent years, and this has motivated the experimenters to study genital herpes especially in connection with pregnancy. The mechanism responsible for possible occurrence of more heavy virus infections (polyomielitis, influenza, genital herpes) in pregnant women than in nonpregnant of the same age, has still not been explained fully. Changed immunity during pregnancy could be an important point. It seems that raised progesterone level is a factor of importance in the depression of cellular immune response during pregnancy^(1, 2), and in addition to that, the effect of progesterone on the vaginal mucous membrane resulting in its increased permeability, stimulates the penetration of the virus into the tissue.

The effect of hormones on the replication of the virus may be considered separately or in connection with changes in the immune response of a pregnant woman to infection. Investigations on the basis of which it would be possible to determine the effect of hormones independently from the immunological mechanisms can only be carried out in vitro.

A large number of factors may affect the replication of the virus in sensitive cells, where importance is also attached to the physiological status of the cells themselves, which are prone to changes under the influence of different hormones. The hormones act on the cell enzymatic systems and thus cause changes in the physiological activity of the cells. The replication of the virus in cells exposed to hormonal influence may be changed, and the changes are mostly quantity-related. If the same virus were then to replicate itself in cells not affected by hormones, there would be no difference in the production of new virus particles in the new cycle⁽³⁾.

The traction of steroid hormones (progesterone) on a cell is presented on the diagram published by Grody *et al.* in 1982⁽⁴⁾.

Steroid hormones act on a large number of different "target" cells, changing in the process their structure and functions, and causing certain general effects, such as induction of the RNA and protein synthesis. The mediators of these effects are specific cytoplasm proteins that bind hormones — the receptors. The binding of the hormones leads to enzymatic or conformational changes in the receptors. Dif-



ferent physical and chemical factors may either activate or inactivate this bond, which is why experimental conditions (temperature, pH, etc.) are important for in vitro investigation.

Hence, it is only logical to expect different results in investigating the effect of hormones on replication of the virus in vitro, since they will largely depend on the type of the cell system in which the virus is replicated. Importance is also attached to the sex of the cells, since the presence of progesterone and estradiol receptors is associated with the action of sexual hormones.

This project involved an investigation into the effect of progesterone on the replication of Herpes simplex virus Type 2 in a culture of Vero cells.

MATERIAL AND METHODS

A continuous culture of Vero cells, grown in glass test tubes, was used for the replication of the virus. The Herpes simplex virus Type 2 (ATCC VR-734) was propagated onto the Vero-cell culture. The titre of the virus amounted to $10^{6.0}$ pfu/ml of suspension.

Progesterone (Mol. weight 314.5) was obtained from Sigma Chemical Co. The hormone was first dissolved in a 95% solution of ethanol, and then in the cell-culture medium, so that final concentration of ethanol amount to 0.05% and 1 ml of the liquid contained 5 micrograms of progesterone.

DESCRIPTION OF EXPERIMENT

A test was made prior to the experiment in order to rule out the possibility of the 0.05% ethanol having a toxic effect on the Vero-cell culture. In this test, the Vero-cells which had been replicating in glass test tubes were exposed to 0.05% ethanol. The cells were kept under observation for several days and it was found that the 0.05% ethanol had no toxic effect in the medium, since no difference in the appearance and growth was noted between the cells replicating in the presence of ethanol.

The first part of the experiment involved the preincubation of the cells with progesterone in the duration of 48 hours. The medium was then drained and the culture inoculated (the inoculum amounted to 0.1 ml). Absorption of the virus was carried out at the temperature of 37 °C and in the duration of one hour, after which the inoculated culture was rinsed with PBS buffer (pH = 7.2-7.4). Replication of the virus was continued in the presence of progesterone at the temperature of 37 °C, and sam-

ples for titration of the virus were taken 10, 21 and 32 hours after inoculation in order to determine the curve of the single-cycle replication of the HSV Type 2 in the presence of progesterone. The control part of the experiment involved a procedure that was identical to the previous one, the only difference being that everything was done in the absence of progesterone.

RESULTS

The results obtained in this experiment are presented on fig. 1, where a curve of single-cycle replication of the virus in the presence of progesterone and a curve of replication in the absence of the hormone were drawn on the basis of the virus titre values obtained at different time intervals after inoculation.

Ten hours after inoculation, there was no difference between the value of the titre of the virus replicated in the presence of progesterone and the titre of control cultures ($10^{2.4}$ pfu/ml). The titre of the virus replicated in the presence of progesterone amounted to $10^{2.7}$ pfu/ml 21 hours

after inoculation, and to $10^{6.1}$ pfu/ml after 32 hours.

The titre of the virus in the control culture amounted to $10^{3.1}$ pfu/ml 21 hours after inoculation, and to $10^{6.1}$ pfu/ml after 32 hours.

It can be seen that there is no significant difference in the production of infectious virus particles during one cycle of replication in the presence of progesterone.

DISCUSSION

The hormones affect differently the various types of virus, and even one and the same virus, which depends not only on the mode of replication of the virus, but also on the cell system in which it is replicated in vitro. Altered replication of the virus in the cells results from changes in their function. There still remains open the question of the direct mechanism whereby the hormone affects the replication of the virus. The following explanations can be regarded as probable:

The effect on the absorption of the virus and changes in the enzymatic systems responsible for the synthesis of the virus nucleic acid and the synthesis of virus specific proteins: the steroid hormones regulate the phosphorylation of the ribosomal basic 5-6 protein, resulting in increased translatory capacity of the ribosomes. Viruses produce the same effect, too.

The action of pregnancy-related hormones has been studied so far individually in vitro, although it is known that the action of progesterone and estrogen is complementary, and even inter-dependent. This fact can serve as an explanation for the similar effect of progesterone and estrogen on the HSV replication.

This project involved the study of the effect of progesterone on the replication of HSV Type 2 in vitro. It has been noted that there is no significant difference in the single-cycle replication between this virus in cells preincubated with progesterone

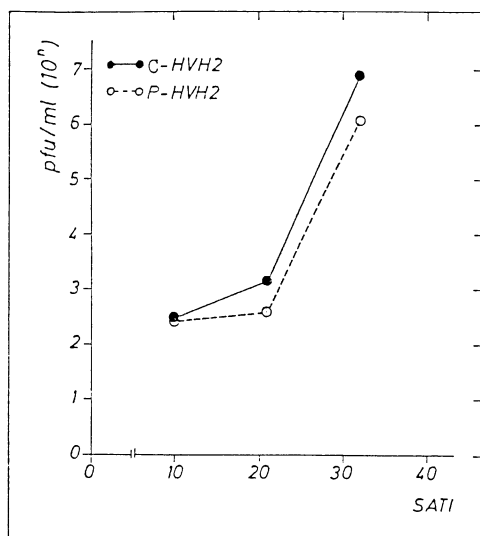


Fig. 1. - Single-cycle replication of the virus in the presence of progesterone (P-HSV 2) and a curve of replication in the absence of the hormone (C-HSV 2).

rone, i.e. in the presence of progesterone, and those in the control culture.

The cell-cultures in which the virus replicated were contained in glass test tubes. Similar results were published by Amstey in 1977⁽³⁾, who made his experiments on the human cells (Wi-38) in plastic tubes.

It is known that due to their physical and chemical properties, plastic materials absorb steroid hormones, especially in the absence of proteins⁽⁵⁾. In our experiment, we used glass test tubes in order to avoid possible loss of progesterone activity resulting from its being absorbed by plastic material. Even so, there was no notable effect of this hormone on the replication of the HSV Type 2, which is in agreement with experiments⁽³⁾. A probable explanation for that is the presence of calf-serum proteins in the medium for the cell culture containing progesterone. In addition, the investigations made by the cited author show that the duration of preincubation of the cells with progesterone is very important, since the latter produced no effect on the replication of HSV when preincubation lasted less than 48 hours.

The effect of hormones on the replication of the virus cannot be generalized on the basis of experiments on one virus only. The inhibiting effect of progesterone was demonstrated in the experiment with vaccinia virus, while it was absent in the case of adenovirus. Other hormones whose activity is enhanced during pregnancy (estriol, human chorionic gonadotropin and human placental lactogen) affect the replication of virus in different ways.

In the experiment with estriol, it was noted that this hormone affects the replication of HSV Type 2 similarly to progesterone, while the human chorionic gonadotropin and human placental lactogen showed a stimulating effect on the replication of this virus. Estriol, human chorionic gonadotropin and human placental lactogen had no effect on the replication of adenovirus⁽³⁾.

In the experiments carried out in the scope of this project, as well as in the experiments carried by other Authors, it is difficult to create in vitro conditions which would be identical to the in vivo ones. This particularly applies to pregnancy due to the accompanying changes in the functions of all organs and organ systems.

CONCLUSION

The effect of progesterone on the replication of HSV Type 2, without the influence of immune mechanisms, was investigated in the scope of this project under in vitro conditions. The results thus obtained showed that there is no significant difference in the production of infections virus particle during one replication cycle in the presence of progesterone.

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