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IMPRINTING WITH OXYTOCIN AND VASOPRESSIN IN CHANG LIVER CELL CULTURESS: HORMONAL OVERLAP, BINDING AND INFLUENCE ON CELL DIVISION

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In Chang liver cells the administration of oxytocin and vasopressin as well as the combined application of the two hormones will result in a positive binding imprinting for oxytocin and a negative binding imprinting for vasopressin. The hormones are able to increase the mitotic capacity of the liver cells even without previous imprinting, both in the case of treatment for 4 hours and for 24 hours; the change, however, is more marked in the case of treatment for 4 hours. Treatment for 24 hours results also in some functional imprinting.

Keywords: oxytocin, vasopressin, hormonal overlap, hormonal imprinting, cell line.

Hormonal imprinting occurs in the perinatal period, at the first contact of the hormone with the developing receptor [3, 4]. Under normal conditions, this event results in the accomplishment of the maturation of the receptors and in the development of the response capacity characteristic of the adult animal. In this period of life the presence in excess of the adequate hormone or the contact with another hormone which, though somewhat different from the adequate one is able to bind to the receptor, leads to abnormal imprinting, a phenomenon which will subsequently manifest itself in abnormal (decreased or increased) binding and response capability of the cell [3, 4, 7]. The phenomenon of hormonal imprinting can be investigated both in unicellulars [6] and in the cell cultures (cell lines) of higher organisms [5]. However, in the latter case the given hormone meets its receptor but not for the first time, the contact having already occurred in the living organism from which the cell line had originated. Nevertheless, the phenomenon of imprinting can be demonstrated and detected also in cell cultures.

The phenomenon of imprinting may be evoked not only with polypeptide [3, 7, 8, 10] and steroid [9] hormones but also with hormones of the amino acid type [12]. The two hormones investigated in the present experiment,

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namely oxytocin and vasopressin, differ from those studied in previous experiments [7] not only because they are oligopeptides consisting of 9 amino acids but also because the difference between the two hormones is minute (only two amino acids and, thus, also their overlapping effect can be investigated).

Materials and methods

In the present experiments Chang liver cells were isolated by trypsine pretreatment and kept in a medium containing 90% Parker 199 and 10% fetal calf serum. Hormonal treatment was applied on the third day.

1. The cells were incubated for 4 hours in media containing either oxytocin (Richter, Budapest) in a concentration of 10^{-6}M or vasopressin (Sandoz, AG, Basel) in a concentration of 10^{-6}M or both hormones in a total concentration of 10^{-6}M. The control group did not receive any hormonal treatment.

Following meticulous washing the cells were cultured in a normal medium for 48 hours. Then one part of the cells was fixed in tubes with methanol and stained according to the May–Grünwald–Giemsa method. The remaining cells were fixed in 4% neutral formol (diluted with PBS) and incubated with fluoresce in isothiocyanate-labelled (FITC, BDH, England) oxytocin or vasopressin for one hour. This procedure was again followed by meticulous washing. The intensity of fluorescence in the cytoplasm and that in the nuclear layer were determined by a Zeiss Fluoval cytofluorimeter; the fluorescence on the nucleus should be determined as well since also the membrane of the nucleus is able to bind hormone [14–17]. The analogous signals of the cytofluorimeter were converted into digital ones by means of an analog-digital processor. The digital signs were recorded by an HP 41C microcomputer and the means, standard deviations, and significance of differences between the groups were computed (Student's t test and analysis of variance). The fluorescence was determined in groups of twenty cells each. The experiments were repeated three times and the results compared to the control, thus each block on the Figures represents the mean of the fluorescence of 60 cells.

2. The cells were kept for 24 hours in a medium containing either oxytocin or vasopressin in a concentration of 10^{-6}M or the mixture of equal amounts of the two hormones. As in the first series, one part of the cultures was fixed for MGG staining, while the other part was meticulously washed and then kept in normal medium for 48 hours. Then a second hormone treatment (in a concentration of 10^{-6}M) was undertaken, while the control group was not given any hormone at all.

Then the cultures were stored in normal medium for further 48 hours, fixed with methanol and stained according to the MGG method.

Both in the first and in the second series of the experiment 1000–1000 cells were counted on the MGG-stained slides by means of a light microscope and the ratio of the mitotic to interphasic cells was determined. Since the experiments were repeated three times blocks of the Figure represent 3000 cells. Student's t test was used for statistical analysis. The mitotic index was expressed in terms of percent of the control.

Results and discussion

Though vasopressin exerts its specific effect on kidney and smooth muscle cells and oxytocin on the cells of the uterus, the two hormones are able to bind to the receptor of other cells, too. Specific vaşopressin receptors were demonstrated by Butlen et al. [1] in the kidney of rats. Collins and Rosengurt [2] provided evidence for the overlapping effect of insulin and vasopressin on Swiss 3T3 cells. They suggested, however, that in this case the effect of insulin might have been exerted through postreceptor mechanisms. Mean-

while Hanif et al. [13] demonstrated the insulin-like activity of oxytocin in adipocytes, and this activity was found to having been exerted through the receptors. On the basis of the above considerations it seemed worth to investigate both the binding to liver cells of vasopressin and oxytocin and the functional imprinting effect exerted on cell division. The latter question is of special interest since vasopressin is a potent mitogenic agent for the Swiss 3T3 cells [2].

In the experiments on binding capacity the liver cells received pretreatment with the hormones for 4 hours. We waited for 48 hours; this time in-

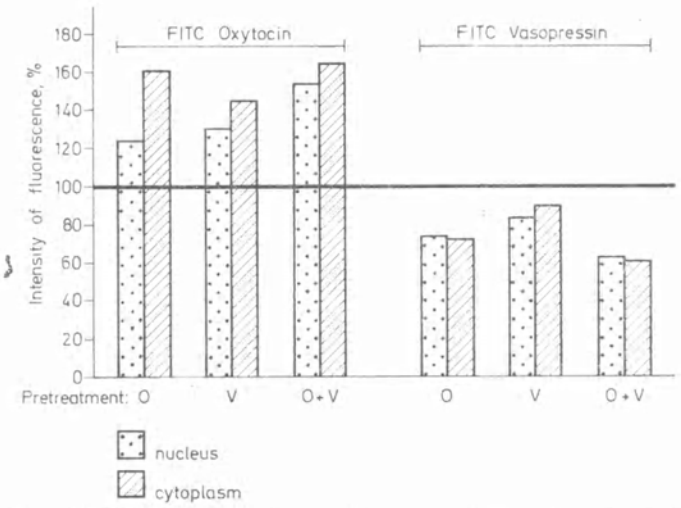

Fig. 1. Binding of FITC-labelled oxytocin and vasopressin to cells pretreated separately or in combination with the hormones

terval seemed to be sufficient enough for either the imprinting to become manifest or the down-regulation in connection with hormone treatment to be eliminated [4, 7.] The binding of the hormone with which the treatment had been carried out as well as that of the other hormone was then studied (Fig. 1). On the basis of the experiments the conclusion may be drawn that treatment with the adequate hormone or with the cognate hormone, as well as the combined treatment with the two hormones led to binding imprinting for oxytocin. Thus, at the second contact with the hormone the binding considerably exceeded the binding seen in those cells which had met the hormone on the culture just for the first time. Meanwhile oxytocin treatment either alone or combined with vasopressin led to negative imprinting for vasopressin, which manifested itself in decreased binding. The effect of vasopressin was similar in tendency but was not statistically significant.

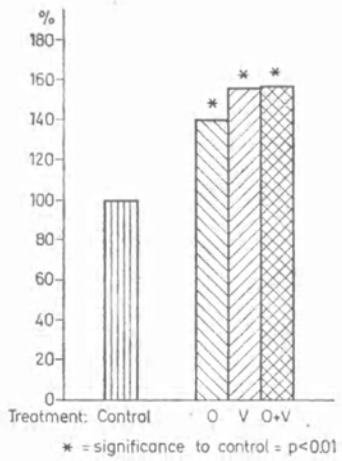

Fig. 2. Effect of hormone treatment for 4 hours on the mitotic activity of Chang cells

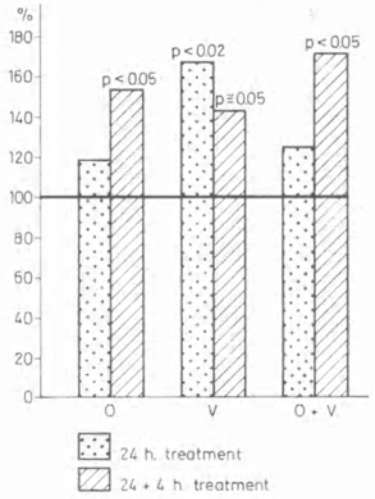

Fig. 3. Effect of treatment for 24 hours and 24 + 4 hours on the mitotic activity of Chang cells

The same hormone treatment for 4 hours — at first contact in the culture — resulted in a considerable increase in the number of mitotic cells (Fig. 2). Though the effect of vasopressin seems to be more marked from this point of view, the difference between oxytocin and vasopressin is not significant statistically.

The number of mitotic cells also increases upon the effect of treatment for 24 hours (Fig. 3). However, both in the case of oxytocin and vasopressin the difference from the control is smaller than that seen following a 4-hour-

treatment. The effect of the combined treatment with oxytocin and vasopressin proved, however, to be more marked in the 24-hour experiment than that following a treatment for 4 hours.

It may be assumed with right that the mitotic capacity of the cells becomes exhausted as result of hormone treatment for excessively long period of time. This assumption may explain the phenomenon that treatment with only one of the hormones (either with oxytocin or with vasopressin) for 24 hours, i.e., a situation where the individual concentration of the given hormone is higher than during combined treatment proved to be less effective than treatment for 4 hours. This is also supported by the phenomenon that while treatment for 4 hours resulted in regular positive and negative binding imprinting both in the case of oxytocin and vasopressin, the imprinting for 24 hours as investigated on the basis of the mitotic index failed to lead to such a marked imprinting for the 4-hour treatment as that seen in the case of binding capacity. Though the number of mitoses is very high also in this case and differs significantly from both the control value and from that obtained with unrepeated treatment (administered for 24 hours only), the difference compared to the results of 4-hour treatment is not significant, although increase or decrease equally be demonstrated, the direction of changes being invariably identical with that seen in the case of hormone binding. When the results obtained in studies on hormone binding and on mitoses are considered together and the alterations of the same direction are evaluated, the conclusion may yet be drawn that not only binding but, consequently, also functional imprinting occurred; however further enhancement of the extent of mitosis was biologically impossible.

Thus, on the basis of these experiments both oxytocin and vasopressin seem to be suitable for evoking hormonal imprinting in Chang liver cells. The two hormones are very close congenes from the chemical point of view and this close relation may explain the phenomenon that both hormones are able to evoke mutual imprinting. This phenomenon was demonstrated in vivo in previous functional experiments [11]. However, the two hormones markedly differ from the point of view that while both oxytocin and vasopressin evoke positive binding imprinting for oxytocin, both hormones evoke negative binding imprinting for vasopressin. On the basis of the above considerations the conclusion may be drawn that in *in vitro* systems oxytocin is characterized by a positive while vasopressin by a negative imprinting effect, as far as the effect on the *adequate* hormone is concerned, while in the case of the overlapping effect it is the quality of the hormone bound after imprinting that determines the direction of the response.

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