IN VITRO STIMULATION OF INITIATION OF POLYTENE REPLICATION CYCLES IN SALIVARY GLANDS OF DROSOPHILA LARVAE BY JUVENILE HORMONE

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Introduction

Ecdysone has been suggested to induce initiation of polytene replication cycles in larval salivary glands in certain Dipteran species (1, 2). On the other hand, in Drosophila it has been seen that during intermoult periods, when the juvenile hormone (JH) titer is high (3), polytene DNA synthesis is more frequent than at the periods of moulting (4, 5) during which ecdysone titer remains high (6). These observations suggest a regulatory effect of these hormones on polytene DNA synthesis. To examine this aspect, we have studied the effects of ecdysone and a juvenile hormone mimic ZR 515 (7) on polytene replication cycles in larval salivary glands of Drosophila nasuta cultured in vitro. The results suggest that while ecdysone does not influence the pattern of polytene replication, JH markedly stimulates initiation of new polytene replication cycles in vitro.

Material and Methods

A modified Poels' tissue culture medium has been used for in vitro culture of larval salivary glands as described earlier (8).

In vitro culture of salivary glands in presence of ZR 515 or ecdysone : Freshly excised sister salivary glands from mid third instar larvae were separated and cultured for different durations (see results) in presence (treated) or absence (control) of ZR 515 or of ecdysone. The dosages of ZR 515 (Zoecon Corporation, California) tested were 3.0 µl, 0.03 µl and 0.0003 µl per ml of medium while that of ecdysone was 2.0 µg and 0.2 µg per ml of medium. Stock solutions of the hormones were prepared by dissolving in 70% ethanol; the hormone were freshly added to the medium at the time of initiation of in vitro culture. The control cultures contained equivalent amount of 70% ethanol. At the end of the culture period the control and treated salivary glands were pulse labelled with ^{3}H-thymidine (Act. 250 µci/ml; Sp. Act. 12.5 Ci/mM, BARC) in fresh medium for 20 min; fixed in 1:3 acetomethanol, squashed and processed for autoradiography (see below).

Another set of sister salivary glands from mid third instar larvae were separated and continuously labelled with ^{3}H-thymidine (2 µci/ml) in presence of ZR 515 (3.0 µl/ml) or of ecdysone (2 µg/ml; Sigma) or in absence of hormone for 24h following which the glands were processed for autoradiography.

ZR 515 treatment of salivary glands from larvae fed on 5-fluorodeoxyuridine (FdU) : Mid third instar larvae (110h after oviposition) were fed on medium containing FdU (200 µg/ml; Sigma) for 24h to block polytene replication cycles at the intersynthetic or early S-period (9). Sister salivary glands from 3-4 FdU fed larvae were separated and cultured in absence (control) or in presence of ZR 515 (3.0 µl/ml) for 2h, 6h, 10h, 20h and 30h following which the glands were pulse labelled with ^{3}H-thymidine as above.

All the above squash preparations were processed for autoradiography with Ilford L4 nuclear emulsion (10). Data were collected from duplicate or triplicate batches of 3-4 sister salivary glands.

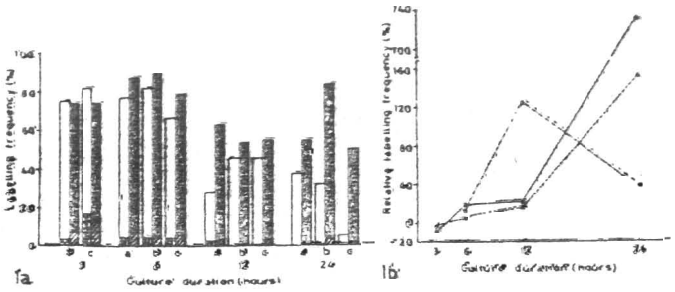
Results

Polytene nuclei at different stages of a replication cycle are characterised by specific patterns of ^{3}H-thymidine labelling (for recent review, see ref 11). During early stages of polytene S-period, ^{3}H-thymidine incorporation is seen in interband and puff regions (interband labelling pattern) followed by the continuous labelling of all the band and interband regions (continuous or C-labelling pattern). Since these stages represent the early part of polytene S-period, together they have been referred to as "early" patterns. The later part of polytene S-period shows discontinuous labelling of specific band regions and are classified into three categories viz., 1D, 2D and 3D. All these together are referred to as "late" patterns. These patterns are seen in replicating salivary gland polytene chromosomes from normally growing D. nasuta larvae (10) and also after in vitro culture of salivary glands (8). Similar patterns of replication were also observed in in vitro ZR 515 or ecdysone treated salivary glands. No qualitative difference in the patterns of replication of the treated and control glands were apparent.

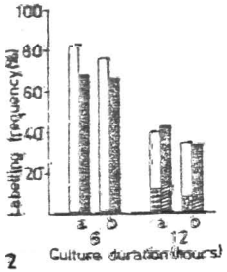
The total frequencies of labelled nuclei as well of nuclei showing early and late patterns of ^{3}H-thymidine labelling in groups of sister control and hormone treated glands are presented in fig. 1a and 2. The relative frequencies of the replicating nuclei in the ZR 515 treated glands in comparison to those in sister control glands are shown in fig. 1b. The results reveal that while the ZR 515 treated glands show higher frequency of replicating nuclei, in ecdysone treated glands the labelling frequencies are not different from those in the corresponding control glands (see fig. 2). The higher frequencies of replicating nuclei in the ZR 515 treated glands are mainly due to increase in the frequencies of nuclei showing late patterns of ^{3}H-thymidine labelling (see fig. 1a).

Glands chronically labelled in presence of ZR 515 show higher frequency of continuous ^{3}H-thymidine labelling and a corresponding decrease in the frequency of late patterns (fig. 3a). In ecdysone treated glands, however, the frequencies of different patterns of labelling after chronic labelling with ^{3}H-thymidine are similar to those seen in sister control glands (fig. 3b).

ZR 515 treatment of salivary glands from FdU fed larvae : To further ascertain the precise nature of the effect of ZR 515 on polytene replication, in vivo FdU treatment has been employed to synchronise the replication cycles in different nuclei of a gland. FdU blocks polytene replication cycles at the early S-period (9). In the present study also, after FdU feeding 70-90% of the replicating nuclei have been seen to be at early stages of polytene S-period (detailed data to be presented elsewhere). In the FdU synchronised glands also, the frequency of different patterns of ^{3}H-thymidine labelling is higher in the ZR 515 treated than in the control glands (fig. 4a). Comparison of relative labelling frequencies of early and late patterns (fig. 4b) shows that the increase in the frequency of labelled nuclei in the 2h, 6h, and 10h treated samples is mainly due to a higher proportion of the early patterns while during the later periods (20h and 30h), the relative frequency of the late patterns is higher in the ZR 515 treated glands.



1a 1b



2

Fig. 1a : ^{3}H-thymidine labelling frequencies in control and ZR 515 treated salivary glands at different time intervals of in vitro culture. a, b and c for each hour represent 0.0003 µl., 0.03 µl and 3.0 µl per ml dose series, respectively, and in each case the left bar represents control and the right bar the treated samples. The height of the bar indicates the total labelling frequency while the sub-divisions show the frequencies of early (control , treated) and late (control, treated) patterns, respectively.

Fig. 1b : Relative frequencies of labelled nuclei in ZR 515 treated glands in comparison to those in sister control glands at different time intervals of culture. The relative labelling frequencies are derived by calculating the difference (increase or decrease) between the total labelling frequencies in corresponding control (taken as 100%) and treated samples shown in fig. 1a. ●—● = 3.0µl ▲—▲ = 0.03µl ■—■ = 0.0003µl per ml.

Fig. 2. ^{3}H-thymidine labelling frequencies in control and ecdysone (e=0.2µg/ml; b=2.0µg/ml) treated salivary glands (for details see fig. 1a).

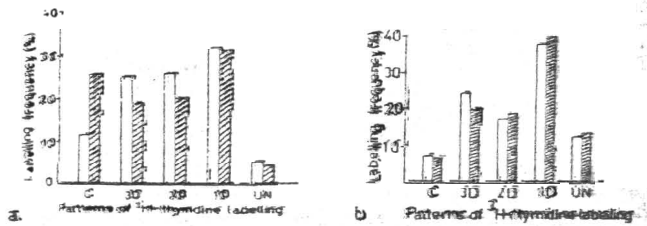


Fig. 3a, b : Frequencies of different ^{3}H-thymidine labelling patterns in glands chronically labelled with ^{3}H-thymidine for 24h in presence (a) of ZR 515 (3.0µl/ml) or (b) of ecdysone (2.0µg/ml). control treated.

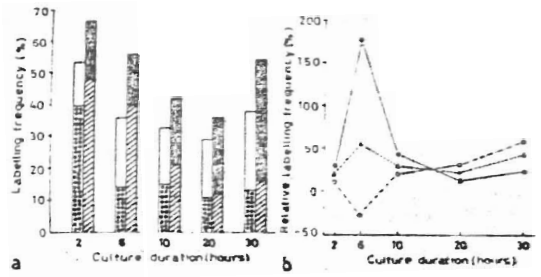


Fig. 4a : ^{3}H-thymidine labelling frequencies in control and ZR 515 (3.0µl/ml) treated FdU-synchronised glands at different time intervals of in vitro culture. For explanation, see fig. 1a.

Fig. 4b. : Relative frequencies of early (●—●) and late (○—○) patterns of ^{3}H-thymidine labelling and of total (▲—▲) labelled nuclei at different time intervals in the ZR 515 (3.0µl/ml) treated glands in comparison to those in control glands seen in fig. 4a. For details, see fig. 1b.

Discussion

The presence of stage specific patterns of ^{3}H-thymidine labelling of the polytene chromosomes during a polytene S-period has been utilized to characterise the effects of ecdysone and juvenile hormone on polytene replication in Drosophila larvae. These hormones regulate growth, differentiation and metamorphosis in Drosophila (3, 12). The method of in vitro analysis adopted in this report has the advantage of precisely monitoring the treatment conditions (viz., defined medium, culture conditions, dose and durations of treatment). Besides, precise control for each treatment was obtained by culturing the sister salivary gland in absence of the hormone since it is known that under normal condition, polytene replication cycles between the two sister salivary glands progress synchronously (13).

Our results show that while ecdysone does not influence the ^{3}H-thymidine labelling patterns and their frequencies, JH brings about a remarkable increase in the frequency of replicating polytene nuclei. The observed increase in the frequency of labelled nuclei at different time intervals of ZR 515 treatment of unsynchronised glands may either be due to a prolongation of the duration of the S-period so that nuclei take longer time to complete their replication cycle and thus appear labelled more often, or this may result from a higher frequency of new initiation of polytene S-period in the presence of ZR 515. The increase in the frequency of late patterns in these glands is more striking since these patterns occupy a longer part of the polytene S-period (9, 11). The results of other experiments support the later alternative. Chronic labelling of salivary glands with ^{3}H-thymidine in presence of ZR 515 shows a specific increase in the frequency of nuclei showing continuous labelling with a corresponding decrease in the frequency of late patterns (3D and 2D) of ^{3}H-thymidine labelling. This would imply that many of these nuclei which were at the late stage of a polytene replication cycle at the beginning of the culture period entered fresh cycles of polytene S-period (interband or C-pattern) so that they finally appear to be continuously labelled. On the other hand, in the control glands in absence of ZR 515, new cycles were not

initiated in these nuclei and thus they continue to show 3D and 2D patterns of ^{3}H-thymidine labelling. Similarly in FdU synchronised glands a greater number of nuclei initiated DNA replication within a short period of in vitro culture in presence of ZR 515. The increase in the frequency of discontinuous patterns in the later periods of culture of FdU synchronised glands further shows that the ZR 515 stimulated new cycles of polytene replication progress to completion in the normal manner.

It is known that the high rate of DNA synthesis in polytene nuclei occurs during intermoult period of larval development (4, 5) and it is during this period that the JH titer remains high (3). Thus the observed stimulation of polytene replication in vitro by the JH mimic is possibly a reflection of the in vivo effect of JH on polytene replication cycle. In this context it is interesting to note that when Drosophila larval salivary glands, which are normally histolysed during pupation, are transplanted to adult abdominal cavity they appear to achieve higher levels of polyteny (14). In adult haemolymph also the JH titer is high (3). It would, therefore, appear that as in in vitro condition, the presence of JH in vivo also facilitates initiation and progression of new polytene replication cycles.

The reduced frequency of polytene nuclei replicating in vivo during moulting of Drosophila larvae (4, 5) could not be simulated in our in vitro study by treatment of salivary glands with high ecdysone concentration. Thus ecdysone titer alone possibly may not cause lowering of the frequency of the replicating polytene nuclei at the time of moulting. Further studies to identify the factor/s which bring about such alteration in the rate of DNA synthesis during different stages of larval development are in progress.

References

1. Amabis, J. M., Cell Differentiation 3 : 199 (1974)
2. Fresquez, C. L., Insect Biochem. 9 : 517 (1979)
3. Riddiford, L. M. and Truman, J. W., In Biochemistry of insects (ed. by M. Rockstein) pp. 308 (1978)
4. Danieli, G. A. and Rodino, F., Nature (Lond.) 213 : 424 (1967)
5. Rodman, T. C., Chromosoma (Berl.) 23 : 271 (1968)
6. Borst, D. W., Bollenbacher, W. E., O'Connor, J. D., King., D. S. and Fristrom, J. W., Dev. Biol. 39 : 308 (1974)
7. Postlethwait, J. H., Biol. Bull. 147 : 119 (1974)
8. Sinha, P. and Lakhotia, S. C., Ind. J. Exp. Biol. 18 : 1059 (1980)
9. Achary, P. M., Majumdar, K., Duttagupta, A. and Mukherjee, A. S. Chromosoma (Berl.) 82 : 505 (1981)
10. Lakhotia, S. C. and Roy, S., J. Cell Sci. 36 : 185 (1979)
11. Mukherjee, A. S. Chatterjee, S. N., Duttagupta, A. K., Chatterjee, R. N., Majumdar, D., Chatterjee, C., Ghosh, M., Achary, P. M. R., Dey, A. and Banerjee, I., In Development and Neurobiology of Drosophila (ed. by O. Siddiqi, P. Babu, Linda M. Hall and Jeffrey C. Hall) pp 57 (1980)
12. Siegel, J. G., and Fristrom, J. W., In The genetics and biology of Drosophila (ed. by M. Ashburner and T. R. F. Wright) pp 317 (1978)
13. Roy, S. and Lakhotia, S. C., Ind. J. Exp. Biol. 15 : 794 (1977)
14. Berendes, H. D., Int. Rev. Cytol. 35 : 61 (1973)