Dissociation of glucocorticoid effects of C-21 steroids at high concentrations in thymocytes[1]

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Summary. A dissociation between inhibition of RNA synthesis and cell lysis was observed when thymocytes of adrenalectomized rats were incubated with high concentrations of pregn-4-ene-11β-ol-3,20-dione and pregna-1,4-diene-11β-ol-3,20-dione. In contrast, no dissociation of these effects was found with the typical glucocorticoids cortisol and corticosterone, nor with their 1,4-diene analogs under the same conditions.

The fact that glucocorticoids cause lymphocytolysis was reported as early as 1944 by Dougherty and White. It is still not clear, however, which of the many early metabolic events elicited by these hormones can be held responsible for the involution of lymphocytes (see Baxter and Rousseau[3] and Roldán et al.[4] for recent reviews). It is generally accepted that the lytic action comprises: a) specific binding of the hormone to a cytosolic receptor and translocation to the nucleus; b) induction of the production of a specific protein; c) inhibition by this protein of RNA and protein synthesis; d) cell lysis[5-7]. However, several aspects of the inhibition of RNA synthesis and of cell lysis, especially those evident at high hormone concentrations, cannot be explained by this sequential hypothesis but better correspond with an alternate mechanism.

We have already reported the inhibition of ^{3}H-uridine incorporation into the perchloric acid precipitate of thymocytes of adrenalectomized rats by cortisol (F), corticosterone (B), pregn-4-ene-11β-ol-3,20-dione (HOP) and their 1,4-diene analogs pregna-1,4-diene-11β,17α,21-triol-3,20-dione (ΔF), pregna-1,4-diene-11β,21-diol-3,20-dione (ΔB) and pregna-1,4-diene-11β-ol-3,20-dione (ΔHOP) in various concentrations[4]. We now correlate this inhibitory response induced by the above mentioned C-21 steroids with their effectiveness to induce cell lysis.

Materials and methods. Minimum essential medium was obtained from Gibco (Grand Island, NY, USA); steroids were obtained as previously described[4].

Incubations of thymocytes from the thymus of adrenalectomized, 90-day-old male and female Sprague Dawley rats, were carried out in triplicate as described[4]. Cell suspensions in minimum essential medium were incubated with and without steroids for 16 h in the presence of 100 U/ml of penicillin and washed once to remove broken cells. Viability was determined by Trypan Blue exclusion. Lysis is defined as 100%-V%, V% being the percent of viable cells compared to controls. Inhibition data refer to 10^7 cells. Percent of inhibition is calculated with respect to controls after a 3 h incubation[4].

Results and discussion. Although HOP and ΔHOP only poorly inhibit uridine uptake at low concentrations (10^{-7} M) after 3 h, a marked decrease in cell number was observed after a 16 h incubation, HOP being slightly more active than ΔHOP (lytic effects with respect to controls, observed after 3 h in the incubations described were within the experimental error; thus data for inhibition of uridine uptake were not affected by cell lysis). At higher concentrations (10^{-6}-10^{-5} M) both cytolytic action and uridine uptake inhibition increased; however, at the highest concentration assayed (10^{-4} M) a dissociation of effects was observed: while inhibition of uridine uptake after 3 h was maximal at this high concentration, the lytic effect after 16 h experienced a drastic decrease to very low values for both steroids (fig.). Typical glucocorticoids like B and F and their 1,4-diene analogs, on the other hand, did not exhibit dissociations of these effects at high concentrations. As can be seen in the table, at 10^{-4} M all of them exhibit high cytolytic action with correspondingly high inhibition of uridine uptake as predicted by the classical mechanism summarized above.

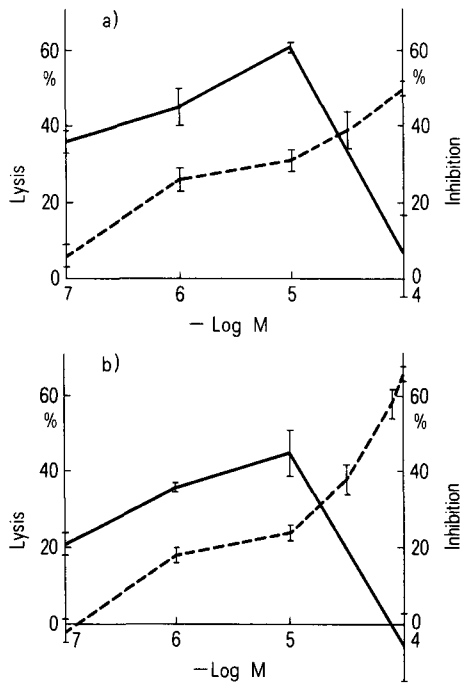
Gelehrter et al.[8] reported a similar dissociation of effects in HTC cells with high concentrations of HOP (10^{-4} M), where a specific nontoxic inhibition of aminoisobutyric acid transport did not correspond with an induction of tyrosine aminotransferase.

The lack of cytolytic activity of both HOP and ΔHOP at 10^{-4} M, is in contrast to the strong inhibition of uridine uptake; particularly the latter steroid had the highest inhib-

Comparison of cell lysis after 16 h and inhibition of ^{3}H-uridine uptake after 3 h by rat thymocytes incubated with high concentrations (10^{-4} M) of typical glucocorticoids and their 1,4-diene analogs

Steroid	% Inhibition[a]	% Lysis
B	43±2 (7)	70±1 (6)
ΔB	51±3 (10)	66±2 (6)
F	45±5 (9)	66±2 (9)
ΔF	38±4 (16)[b]	74±2 (6)

Number of assays in parentheses. [a]Inhibition data, taken from Roldán et al.[4], are referred to 10^7 cells. [b]High dispersion was observed between experiments in the responses to ΔF.



Correlation between cell lysis (-) and inhibition of ^{3}H-uridine uptake (--) by rat thymocytes incubated with different concentrations of *a* HOP and *b* ΔHOP. (Inhibition data, taken from Roldán et al.[4], are referred to 10^7 cells.) Each point corresponds to at least 6 assays.

itory effect of all the hormones tested by us[4]. This suggests that these C-21 steroids, which lack the 21 hydroxy group as a minimun requirement for a steroid to provoke lymphocytolysis[9], exhibit special structural characteristics allowing them, specifically at high concentrations, to inhibit RNA biosynthesis. Hence these steroids at these concentrations may act by a different mechanism, including a 'membrane effect'[8,10]. This alternate mechanism would not lead to cell lysis.

1 Acknowledgments. This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina to the Instituto de Biologia y Medicina Experimental and the Programa de Regulación Hormonal y Metabólica as well as by financial help from the Secretaría de Ciencia y Tecnología.

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0014-4754/83/060617-02$1.50 + 0.20/0

Juvenile hormone levels, vitellogenin and ovarian development in *Acheta domesticus*

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Summary. Juvenile hormone hemolymph titres have been determined by radioimmunoassay at the beginning of imaginal life in the cricket *Acheta domesticus*. JH III levels increased during previtellogenesis and at the onset of vitellogenesis in the successive waves of ovocytes. Vitellogenin synthesis started between the 24th and 40th h of imaginal life after bursts of juvenile hormone production and before any ecdysteroïd had appeared in either the hemolymph or the ovaries.

Hormonal control of ovarian development and vitellogenin synthesis in insects have been studied by several authors[1-4]. In most species, reproduction is controlled by juvenile hormone which acts both on vitellogenin synthesis and ovocyte maturation. In numerous species, the stimulation of vitellogenin synthesis has been demonstrated in vivo, for example in *Leucophaea maderae*[5,6], *Leptinotarsa decemlineata*[7] and *Locusta migratoria*[8,9]. However, up to now only Wyatt and al.[10] were able to stimulate vitellogenin synthesis in vitro in *Locusta*. In *Aedes*, the concept that 20-hydroxyecdysone directly stimulated vitellogenin synthesis by fat body cells previously exposed to juvenile hormone has been supported by Fallon and al.[11] and Hagedorn and al.[12]; recent findings have, however, failed to confirm earlier observations[13]. In *Anopheles*[14], as in *Drosophila*[15,16] and in *Oncopeltus*[17], injections of ecdysterone did lead to the appearance of vitellogenin in the hemolymph. Induction of vitellogenin synthesis by ecdysone alone has been reported in *Sarcophaga bullata*[18,19]. Nevertheless, it seems that in Diptera both juvenile hormone and ecdysteroïds are involved in the hormonal regulation of yolk protein synthesis[20]. Juvenile hormone is the hormone controlling vitellogenesis, since it not only produces morphological changes in follicular cells of ovocytes[21-25], but also stimulates the production and uptake of vitellogenin by maturing ovocytes[26].

In the Orthoptera, *Locusta* has been the most widely used by insect endocrinologist, but in a few studies *Acheta domesticus* has been used. Yolk proteins have been studied in the house cricket by Kuntz and Petzelt[27] and Bradley and Edwards[28]. Female specific proteins have been identified both in the hemolymph of adult crickets and in the maturing ovocytes[29], and modifications of neurosecretory material in the course of ovarian maturation have been described[28]. But nothing was known about the changes in juvenile hormone during oogenesis in this species.

In a previous study[30], we showed that in *Acheta* some ecdysteroid production occurs in the ovary and variations

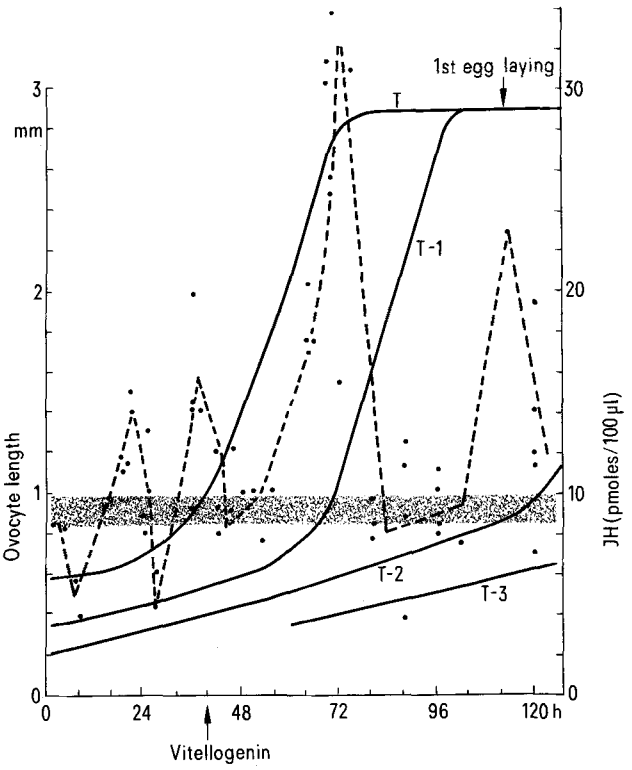

Figure 1. Hemolymph JH titre and ovocyte growth. Abscissae: age of female imagos. Ordinates: JH titres in pmoles/100 µl (---); ovocyte lengths in mm (—), T_1 lengths of terminal ovocyte; T-1, T-2, T-3, lengths of ovocytes from preceding ranks. Hatched area corresponds to the length at which the patency of the follicular spaces appears. Vitellogenin appears in the hemolymph at the time indicated. The first egg laying occurs at the time indicated by an arrow.