Inhibition by tryptophan of nucleolar RNA synthesis in salivary gland nuclei of *Chironomus thummi*

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Summary. Tryptophan, but not glutamine and lysine, inhibits the incorporation of ^{3}H-uridine into nucleoli of explanted salivary glands by about 60%. In isolated nuclei also, the nucleolar incorporation of ^{3}H-uridine triphosphate is reduced to about 50% by tryptophan. It is concluded that tryptophan acts directly at the nuclear level and the possible mechanisms involved are discussed.

Tryptophan elicits specific changes in the puffing pattern of polytene chromosomes in explanted salivary glands of *Chironomus thummi*[2]. The tryptophan-induced puffs synthesize RNA, as evidenced by the incorporation of ^{3}H-uridine[2]. In experiments with isolated polytene chromosomes, Robert and Gopalan[3] have shown that the effects of tryptophan on puffing can be attributed to a direct action of the amino acid at the chromosomal level. The amino acids were found to influence the structural state of the bands in isolated chromosomes. Tryptophan, in particular, was able to elicit specific responses on individual bands. The purpose of the present study was to investigate the effects of amino acids on nucleolar RNA synthesis. Our results show that tryptophan inhibits nucleolar RNA synthesis in both explanted salivary glands and isolated nuclei. From these results we conclude that tryptophan, as in the case of its effects on puffing, affects the nucleolar RNA synthesis by direct interaction with nuclear constituents.

Material and methods. Mid-4th instar larvae (morphological stages 2-4 after Kroeger[4]) of a laboratory bred strain of *Chironomus thummi* were used in the present study. Carefully explanted salivary glands were incubated on siliconized slides under paraffin oil for 2 h at 20-22 °C. The incubation medium (PM) was made up of 2 mM KCl, 28 mM disodium fumarate, 5 mM $CaCl_2$, 28 mM NaCl, 7 mM magnesium succinate, 5 mM TES (tris- (hydroxymethyl)-methyl-2 amino ethane-sulfonic acid-sodium salt, pH 7.4) and with either 80 mM L-tryptophan or 80 mM glutamine[5]. 1 of the 2 salivary glands from a larva was incubated in 15 µl of PM with tryptophan and the sister gland in PM with glutamine. After 90 min, a drop (0.5 µl) of ^{3}H-uridine (NEN Chemicals GmbH, Frankfurt; sp. act. 25.7 Ci/mM) was added and the glands were incubated for a further period of 30 min, after which they were fixed, washed, stained, squashed and processed for autoradiography.

Salivary gland nuclei were isolated by a method developed by Robert[6]. In brief, the method consisted of the following steps: a) treatment of explanted glands at 2-4 °C in a modified Ringer solution (87 mM NaCl, 3.2 mM KCl, 1.3 mM $CaCl_2$. 1 mM $MgCl_2$ and 10 mM tris-maleate pH 6.3) containing 10 mg/ml digitonin, b) isolation of nuclei by pipetting the glandular suspension with micropipettes of specific diameter, c) washing the isolated nuclei 3 times in Ringer solution (pH 7.3, 10 mM tris-HCl buffer). Nuclei isolated in this manner were transferred by micropipettes to incubation chambers (cavities of 3-4 mm diameter in a parafilm layer heat sealed onto microscope slides). The Ringer solution was replaced by the incubation medium (IM) which was made up of 150 mM KCl, 45 mM NaCl, 10 mM $MgCl_2$, 25 mM TES (tris- (hydroxymethyl)-methyl-2 aminoethane-sulfonic acid-sodium salt, pH 7.5), 6 mg/ml glutathione, 2 mg/ml each of ATP, GTP, CTP and 12 µ C/ml ^{3}H-UTP (NEN Chemicals GmbH, Frankfurt; sp. act. 19.8 Ci/mM).

A mass preparation of isolated nuclei (from 30 glands) was divided into 2 parts, 1 of which was incubated in IM with 80 mM tryptophan and the other in IM without amino acids. After 60-min incubation, nuclei were fixed and washed in 5 changes of ice-cold acetic acid (45%), stained in acetoorcein, squashed and processed for autoradiography.

The intensity of labelling over nucleoli was estimated by determining the loss in intensity of the transmitted light with the help of a Zeiss photomicroscope equipped with a spot-light measuring device. The light intensity was measured only in intact nucleoli still attached to the organizer at 3 different regions (300 µm^2 each) and compared with that measured in free regions of the autoradiogram (background only): 20 nucleoli were measured in each preparation. The loss of light intensity was found to be proportional to the grain density; this was established by grain counting in the same region where the light intensity was measured. The data was statistically analysed.

Results. When explanted salivary glands are incubated in the presence of tryptophan, the incorporation of ^{3}H-uridine into nucleoli is markedly reduced as compared to that in nucleoli of sister glands, which are incubated under similar conditions in glutamine (figure 1; table 1). The mean value for the incorporation in the presence of tryptophan is 40% of that in the presence of glutamine. No differences are observed in nucleolar incorporation of glands incubated in PM medium with glutamine, lysine or without amino acids (PM alone or hemolymph).

In order to test if the inhibition of nucleolar RNA synthesis by tryptophan is mediated by any cytoplasmic factors or events, we investigated the effects of this amino acid on

Table 1. Incorporation of ^{3}H-uridine into nucleoli of explanted salivary glands of *Chironomus thummi*

Incubation medium	^{3}H-uridine incorporation*
PM** + glutamine	56.85 ± 1.61 (n = 204)
PM + tryptophan	22.85 ± 1.15 (n = 165)

* The intensity of ^{3}H-uridine labelling is expressed as loss of intensity of the transmitted light (%) which was found to be proportional to grain density (see methods). The values given are means of n-measurements ± SEM. The 2 mean values are different at a 0.01 level of significance. ** For composition of PM medium see methods.

Table 2. Incorporation of ^{3}H-UTP into nucleoli of isolated salivary gland nuclei of *Chironomus thummi*

Incubation medium	Incorporation of ^{3}H-UTP*
IM** − tryptophan	46.56 ± 0.87 (n = 474)
IM + tryptophan	22.28 ± 0.54 (n = 366)

* The intensity of ^{3}H-UTP incorporation is expressed as loss of intensity of the transmitted light (%) which was found to be proportional to grain density (see methods). The values given are means of n-measurements ± SEM. The 2 mean values are different at a 0.01 level of significance. ** For composition of IM medium see methods.

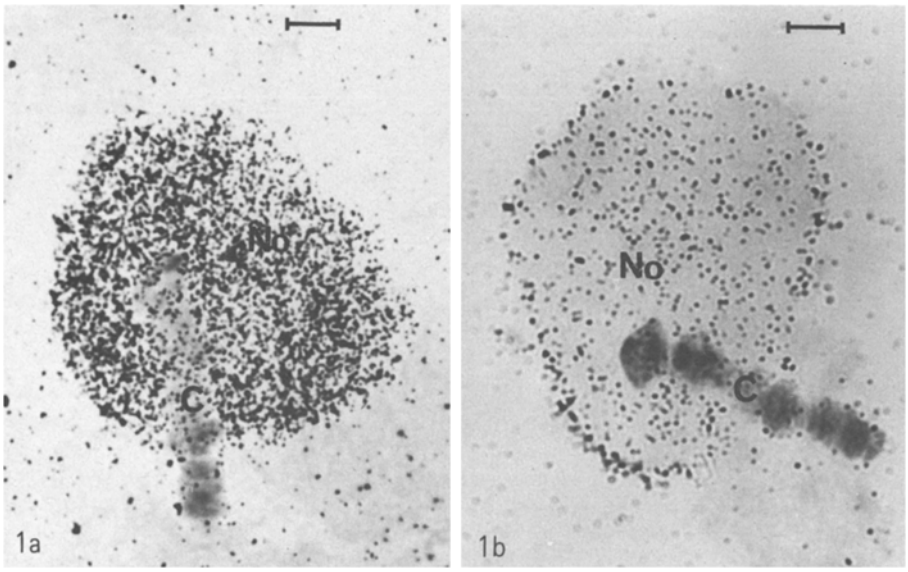

Fig. 1. Incorporation of ^{3}H-uridine into nucleoli of explanted salivary glands of *Chironomus thummi* in the presence of a) 80 mM glutamine and b) 80 mM tryptophan. For composition of incubation medium (PM) see methods. Nucleoli in *a* and *b* are from sister glands. The intensity of labelling in *a* and *b* corresponds to the mean values in table 1. Aceto-orcein squash. No = nucleolus, C = chromosome IV. Magnification: bar denotes 10 µm. × 1010.

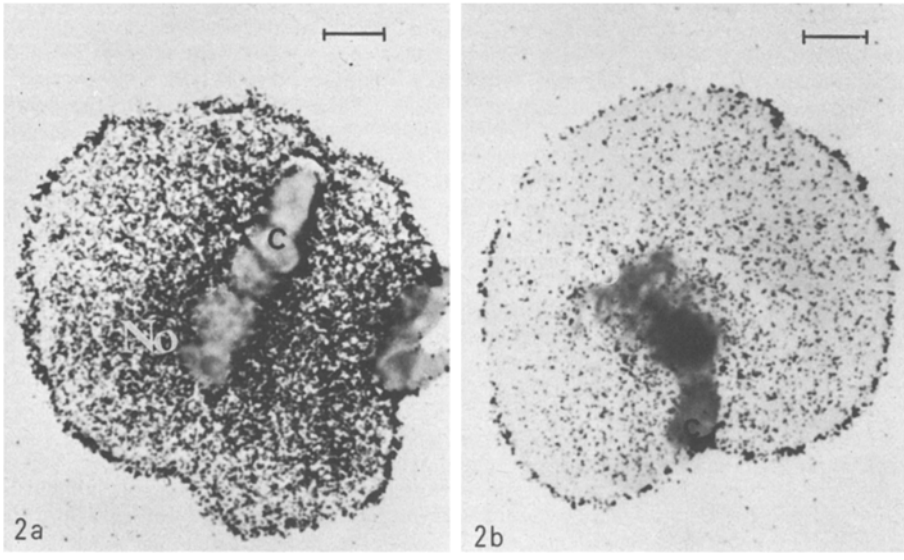

Fig. 2. Incorporation of ^{3}H-UTP into nucleoli of isolated salivary gland nuclei of *Chironomus thummi* a) in the absence and b) in the presence of 80 mM tryptophan. For composition of medium (IM) see methods. The intensity of labelling in *a* and *b* corresponds to the mean values in table II. Aceto-orcein squash. No = nucleolus, C = chromosome IV. Magnification: bar denotes 10 µm. × 840.

nucleolar RNA synthesis in isolated salivary gland nuclei. Nuclei isolated by our method have been shown to actively synthesize nucleolar RNA even in the absence of exogeneous RNA polymerase[6]. Isolated nuclei were incubated in the IM medium (for composition see methods) with or without 80 mM L-tryptophan. As in explanted glands, tryptophan causes a marked reduction in the incorporation of ^{3}H-uridine triphosphate into nucleoli (figure 2; table 2). The mean value of incorporation in the presence of tryptophan is about 50% of that in controls.

Discussion. Tryptophan exhibits differential effects on chromosomal and nucleolar RNA synthesis in the salivary glands of *Chironomus*. Chromosomal RNA synthesis is enhanced, as evidenced from its effects on puffing and incorporation of ^{3}H-uridine in polytene chromosomes[2]. On the other hand, nucleolar RNA synthesis is markedly inhibited by tryptophan both in explanted glands and isolated nuclei.

Both stimulatory[7-10] and inhibitory[11] effects of amino acids on synthesis of DNA-like and ribosomal RNA, and on the activity of Mg^{2+}-stimulated RNA polymerase, have been reported. In particular, tryptophan enhances both ribosomal[9,10] and DNA-like[8,9] RNA synthesis, as well as the activity of Mg^{2+}-stimulated RNA polymerase[11] in rat liver. Our results clearly indicate a suppression of nucleolar RNA synthesis in salivary gland nuclei of *Chironomus* by tryptophan.

The fact that the inhibitory effect manifests itself both in isolated nuclei as well as in explanted glands, suggests that no cytoplasmic factor or event is involved. As in the case of tryptophan-induced changes in puffing[2], where tryptophan has been shown to act directly at the chromosomal level[3], its effect on nucleolar RNA synthesis is probably due to a direct action. In another paper[3], we have presented a detailed discussion on the possible mechanisms of interaction between amino acids and chromosomal constituents. Since specific interactions between tryptophan and DNA are possible by intercalation of the amino acid between the bases[12,13] which leads to conformational changes in the structure of the nucleic acid[14], we presume that tryptophan acts by altering the availability of the DNA template that codes for nucleolar RNA.

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2 H.N.B. Gopalan, Chromosoma *44*, 25 (1973).
3 M. Robert and H.N.B. Gopalan, Kenya Nat. Acad. J. Sci. and Technol., in press (1979).
4 H. Kroeger, Chromosoma *15*, 36 (1964).
5 A.L. Politoff, S.J. Socolar and L.R. Lowenstein, J. gen. Physiol. *53*, 498 (1969).
6 M. Robert, Chromosoma *36*, 1 (1971).
7 M.T. Franze-Fernandez and A.O. Pogo, Proc. natl. Acad. Sci. USA *68*, 3040 (1971).
8 C.N. Murthy and H. Sidransky, Biochim. biophys. Acta *262*, 328 (1971).
9 M. Oravec and A. Korner, Biochim. biophys. Acta *247*, 404 (1971).
10 J. Vesely and A. Cihak, Biochim. biophys. Acta *204*, 614 (1970).
11 R. Shields and A. Korner, Biochim. biophys. Acta *204*, 521 (1970).
12 G. Arcaya, N.E. Pantoja, M. Pieber, C. Romero and J.C. Toha, Z. Naturforsch. *26b*, 1026 (1971).
13 J.L. Dimicoli and C. Helene, Biochimie *53*, 331 (1971).
14 C. Helene, Nature, New Biol. *234*, 120 (1971).

Microperoxisomes in steroidogenic cells of the rat ovary: Interstitial, thecal and luteal cells[1]

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Summary. Microperoxisomes are present in luteal, interstitial and thecal cells. They are in close relation with smooth endoplasmic reticulum and lipids. Their probable role in steroid biosynthesis is discussed.

Peroxisomes (or microbodies) are found in a variety of plant and animal cells[2,3]. Biochemically peroxisomes are characterized by the presence of oxidative enzymes generating H_2O_2 and catalase, which are able to break down H_2O_2[4].

Morphologically, by electron microscopy, they were originally noted in the cells of the proximal convoluted tubule of the kidney[5] and in liver cells[6,7]. In these cells they appear as rather round organelles of about 0.5–1.0 µm in diameter, bounded by a unit membrane and contain a dense matrix in which a cristalline nucleoid is evident. Although, morphologically, it is sometimes difficult to differentiate them from primary lysosomes, cytochemically they can be easily distinguished from lysosomes because they lack acid phosphatase.

More recently, smaller peroxisomes (0.1–0.45 µm in diameter) have been described in a large variety of tissues. These organelles, considered by some authors as progenitors of the larger peroxisomes[8], are commonly called 'microperoxisomes' but lack a central nucleoid and may sometimes be easily confused with primary lysosomes. Cytochemically they can be identified as microperoxisomes because they do not contain acid phosphatase as do lysosomes, and better because they can be easily identified by their specific staining with 3–3′ diaminobenzidine (DAB)[9].

More recently, a number of microperoxisomes have been identified in a number of steroid-secreting cells and their possible significance in steroid biosynthesis has been discussed.

Among steroidogenic tissues studied, microperoxisomes have been cytochemically identified in adrenal cortex[10–12], in Leydig cells[13–15] and in corpus luteum of late pregnancy[16–18]. In all these cells, they have been reported as frequently being closely associated with membranes of smooth endoplasmic reticulum. The purpose of this paper is to identify, by DAB staining, microperoxisomes in steroidogenic cells of adult ovary (luteal, thecal and interstitial cells) and to describe their possible relationship with other cellular organelles.

Materials and methods. Morphology. 5 anesthetized healthy young adult albino rats were used in this study. Ovaries were perfused with a solution of 3% glutaraldehyde buffered to pH 7.4 with 0.1 M sodium cacodylate for 4–6 h at 4 °C.

The tissues, cut into small pieces, were then washed in the same buffer, postfixed in 1.0% osmium tetroxide, for 1–2 h, dehydrated in alcohol, and embedded in Epon 812[19]. Thin sections cut on a Porter-Blum MT-1 ultramicrotome were stained with uranyl acetate[20] followed by lead citrate[21] and examined in a Zeiss EM9A electron microscope.

Cytochemistry. Small pieces of tissue (1–2 mm^3) were fixed for 4–6 h in 3% glutaraldehyde buffered to pH 7.4 with 0.1 sodium cacodylate, then rinsed overnight in the same buffer at 4 °C. For cytochemical identification of microperoxisomes, the pieces were incubated in DAB oxidation medium at pH 9.0[9]. In the control experiments, the pieces were incubated in medium lacking H_2O_2.

In other control tissue, both the preincubation and incubation medium contained 0.2 M aminotriazole. After incubating in the medium with DAB, the pieces were washed 3 times (5 min each) in 0.05 M 2-amino-2-% methyl-1,1-propandiol buffer (pH 9.0), postfixed in 1% cacodylate buffered osmium tetroxide for 1 h, dehydrated in a graded series of alcohol, and embedded in Epon 812. Thin sections, either unstained or stained with uranyl acetate and

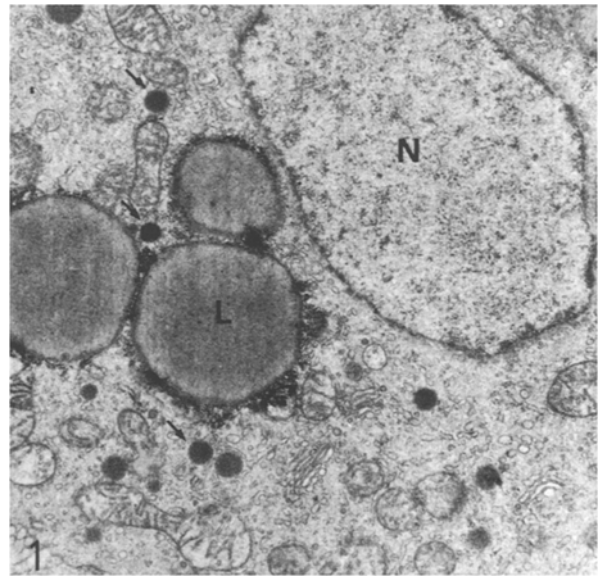

Fig. 1. Rat's ovary: corpus luteum. N, nucleus; L, lipids droplets; arrows, microperoxisomes.