REVISTA ESPAÑOLA DE FISIOLOGIA, 37, 485-490. 1981

Effect of Narciclasine and Puromycin on the Synthesis of Rapidly Labelled RNA in Excised Salivary Glands of *Chironomus thummi*

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(Received on April 22, 1981)

P. ALLER. *Effect of Narciclasine and Puromycin on the Synthesis of Rapidly Labelled RNA in Excised Salivary Glands of* Chironomus thummi. Rev. esp. Fisiol., 37, 485-490. 1981.

The effect on transcription of two protein synthesis inhibitors, narciclasine and puromycin, when applied in short periods of time to explanted salivary glands of *Chironomus thummi*, has been studied. Narciclasine (6 μM) enhances the uptake of uridine and stimulates the synthesis of giant-heterogeneous, pre-ribosomal (38 S) and low-molecular weight (4-5 S) RNA. However, puromycin (200 μM) inhibits uridine uptake and decreases selectively the synthesis of pre-rRNA, while transcription of low-molecular weight RNA is stimulated. These effects are compared with those previously found to be produced by other inhibitors of translation in this biological system.

Narciclasine, an alkaloid which accumulates in *Narcissus* sp. bulbs, has been characterized as a powerful inhibitor of protein synthesis in eukaryotic cells, by preventing peptide bond formation (21). Moreover, it has been shown that narciclasine exerts an antimitotic action in animal (5) and plant (19) cells, and accelerates nucleologenesis in plan meristematic tissues (19). Beyond these effects, very little is known about modifications of cell metabolism caused by this drug.

The purpose of this work is to give some information of the effect of narciclasine (NC) and puromycin (PR) on transcription, when they are applied in short periods of time to excised salivary glands of *C. thummi*. Firstly, the action of these drugs on uridine uptake and the incorporation of this nucleoside into total RNA is studied. Then, the changes in the synthesis of various rapidly labelled RNA types are analyzed by electrophoresis.

Abbreviations: hnRNA, heterogeneous RNA; pre-rRNA, precursor of ribosomal RNA; NC, narciclasine; PR, puromycin; EDTA, ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulfate: TCA, trichloroacetic acid.

Materials and Methods

Biological. Salivary glands from late 4th instar larvae of *C. thummi* were used

in all the experiments. The stock of *C. thummi* was reared from animals collected in Valencia (Spain). The larvae were cultured in the laboratory, as described previously (2).

Chemicals. DL-(4,5 [n]-^{3}H) lysine monohydrochloride (specific activity 10 Ci/mmole) and (5,6-^{3}H) uridine (specific activity 40 Ci/mmole) were purchased from Amersham. Narciclasine was prepared and kindly donated by Dr. A. M. Selman. Puromycin was obtained from Serva Feinbiochemica, agarose C from Hispanagar (Madrid, Spain), Soluene 350 from Packard Instruments, and PPO and POPOP from Intertechnique (France).

Drug treatment. Usually, six pairs of glands were used in each determination. After being explanted, the glands of each pair were separated to form two groups. One of them was incubated in about 1 ml of *Chironomus ringer* (C.R.) solution (18) or Cannon's modified medium (17) containing NC or PR. The other group, serving as control, was incubated in the same media without the drugs. As NC has to be dissolved in absolute ethanol, incubation media with this drug also contained small concentrations of ethanol (from 0.0025 % to 0.25 %). In all cases, the corresponding amount of ethanol was also added to the medium for control glands.

Measurement of amino acid incorporation. Excised glands were labelled for 10 min with 50 μM (^{3}H)-lysine in C.R. solution. The isotope was then removed and the incorporation stopped by adding 10 % cold TCA containing 0.1 % of non-radioactive lysine. The relative rate of (^{3}H)-lysine incorporation into proteins was measured as the acid-precipitable radioactivity after digestion of the labelled glands in OHNa, as previously described (8).

Measurement of uridine uptake and incorporation. Excised glands were labelled for 10 min with 50 μM (^{3}H)-uridine in Cannon's medium. After washing the glands with a buffered saline solution, the relative rate of uridine uptake into the cells was estimated by measuring the amount of acid-soluble radioactivity extractable by TCA, following the method described in a previous work (2). The relative rate of uridine incorporation into RNA was evaluated as the acid-precipitable radioactivity measured after digestion of the labelled glands in a pronase-containing solution (8). More than 98 % of the incorporated radioactivity corresponded to RNA, as was revealed by enzymatic treatments (data not shown). To determine the effect of NC or PR on transcription, the acid-precipitable counts in drug-treated glands were corrected for the effect of these agents on (^{3}H)-uridine uptake into the cells. The procedure was essentially that described by other authors (11, 20) for cultured mammalian cells. Namely, the ratio of acid-precipitable counts in drug-treated glands to the corresponding control glands was multiplied by the inverse ratio of acid-soluble counts. This product might give an estimate of RNA synthesis in treated vs. control glands, if no alteration in the processing of uridine occurs after its entry into the cell, and if there is no uridine pool compartmentalization (20).

Gel electrophoresis of labelled RNA. Labelled RNA, extracted with a pronase-containing solution, was precipitated over night with 2 vol. ethanol, 0.3 M NaCl, at —20° C. Non-radioactive RNA from *Drosophila melanogaster* was added as a carrier. The precipitates were recovered by centrifugation (10 min at 12,100 *g*) and dissolved in 100 μl of electrophoresis buffer consisting of Tris (10.9 g), disodium EDTA (0.93 g), boric acid (5.5 g), SDS (1 g) and distilled water to 1 l, at pH 8.4. The fractionation of the RNA was carried out in 1 % agarose disc gels (8). Processing of gels for radioactivity counting was essentially performed as described by Ed-

STRÖM and TANGUAY (9). Counts in gels containing RNA from drug-treated glands were adjusted for changes in the rate of (^{3}H)-uridine uptake, essentially as above.

Results

To test the efficiency of NC as inhibitor of protein synthesis in this biological system, the activity or (^{3}H)-lysine incorporation in glands pre-incubated with increasing concentrations of the drug was firstly determined. The results in figure 1 indicate that amino acid incorporation was highly sensitive to NC, since this almost completely inhibited the process at a dose of 3 μM. A considerably higher concentration of PR (200 μM) is necessary to obtain a similar degree of inhibition (10), and personal observations).

Modification of uptake and incorporation of uridine. The effect of NC and PR on the activity of uridine incorporation into total RNA is indicated by the results shown in table I. The incorporated radioactivity was considerably enhanced

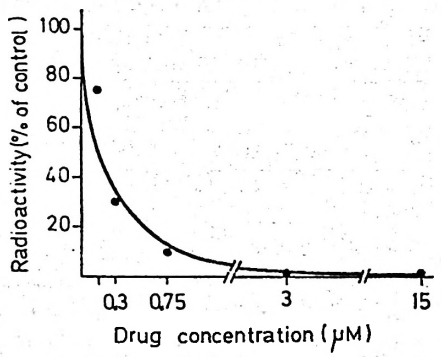

Fig. 1. *Effect of NC on lysine incorporation.* Glands were incubated for 40 min in CR medium containing different concentrations of NC and then briefly labelled with [^{3}H]-lysine in the presence of the drug. The radioactivity incorporated into proteins was determined and expressed as percentage of the untreated sister glands.

Table I. *Effect of narciclasine (NC) and puromycin (PR) on uridine uptake and incorporation.*

Glands were incubated for 1 h in Cannon's medium containing 6 μM of NC or 200 μM of PR, and then briefly labelled with [^{3}H]-uridine in the presence of the inhibitor. Uridine uptake and incorporation of this nucleoside into total RNA were estimated and expressed as percentages of the untreated sister glands. Values represent the mean ± S.E.M. of three determinations.

	% of control		
Inhibitor	«Uncorrected» incorporation	Uptake	«Corrected» incorporation
NC	289±37	177±18	154±12
PR	26± 3.3	29± 0.6	91± 8

by NC and decreased by PR. It could be that at least part of these modifications were a reflection of changes affecting the rate of nucleoside uptake. In fact, this process appeared to be stimulated by NC and inhibited by PR (table I). When incorporation values were corrected for alterations affecting the precursor uptake, the activity of uridine incorporation seemed to be scarcely modified by PR, although it still remained higher in NC-treated gland cells than in controls.

Modification of the synthesis of different RNAs. To analyze the effect of NC and PR on the synthesis of individual RNA types, radioactive samples of glandular RNA were fractionated by electrophoresis. Three components can be discerned in the radioactivity profiles (figs. 2 and 3), which correspond to the three main types of rapidly labelled RNA in dipteran polytene cells (7): a prominent peak, which represents the first nucleolar transcript (pre-rRNA 38 S); a smaller peak, which includes the low-molecular weight (4-5 S) RNA; and the giant-hnRNA (40-100 S), located prior to the pre-rRNA peak. It was found that, after 1 h of treatment, the activity of uridine incorporation

into all these RNAs was higher in NC-treated gland cells than in controls (fig. 2, table II). However, with a 3 h treatment the incorporation into giant-hnRNA dropped to the control level. On the other hand, the administration of PR produced a quite different pattern of modifications (fig. 3, table II), in such a way that only the incorporation into low-molecular weight RNA was slightly enhanced, while incorporation into pre-rRNA was lower than in controls. Incorporation into giant-hnRNA remained practically unchanged. This differential action of PR on the synthesis of the discerned RNA types could explain the lack of effect of the drug on total RNA transcription (table I).

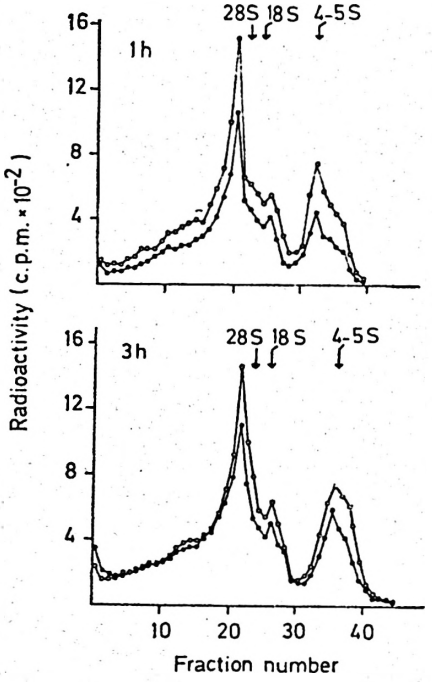

Fig. 2. *Electrophoretical analysis of RNA from NC-treated glands.*
Glands were incubated for 1-3 h in Cannon's medium containing 6 μM of NC and then briefly labelled with [^{3}H]-uridine in the presence of the drug. The nucleic acids were extracted and separated by electrophoresis, and the radioactivity profiles obtained. As control, the sister glands were incubated and labelled in the absence of the drug. ●—●, control; ○—○, NC. Counts in profiles from NC-treated glands were corrected for changes in the rate of uridine uptake. Arrows indicate the position of the peaks of the marker RNA, in U.V. light.

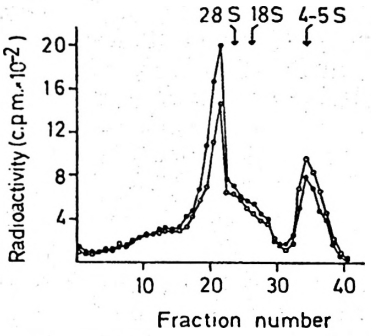

Fig. 3. *Electrophoretical analysis of RNA from PR-treated glands.*
The incubation period was of 1 h. ●—●, control; ○—○, PR (200 μM). Experimental conditions, as in figure 2. Counts in profiles from PR-treated glands were corrected for changes in the rate of uridine uptake.

Table II. *Effect of narciclasine (NC) and puromycin (PR) on the synthesis of different rapidly labelled RNAs*
Values were obtained from spectra in figures 2 and 3. Radioactivity in each type of RNA was measured in profiles from drug-treated glands and expressed as percentage of the value in the correspondent control profile. Radioactivity in the pre-rRNA (38 S) species was measured as the fraction of the peak which emerge from the heterogeneous RNA background.

		% of control		
Inhibitor	Hours of treatment	Giant hn RNA	pre-rRNA	4-5 S RNA
NC	1	146	145	176
	3	96	160	148
PR	1	94	65	121

Discussion

The results reported in this work indicate that NC and PR, when applied in short periods of time to excised salivary glands of *C. thummi*, modify in a different manner uridine uptake and the production of various rapidly labelled RNA types. NC stimulates uridine uptake and enhances the incorporation of this nucleoside into giant-heterogeneous, preribosomal and low-molecular weigth RNA. These effects are essentially coincident with those previously found to be provoked by other inhibitors of translation — cycloheximide (35 μM), anisomycin (75 μM) and emetine (80 μM) — when used under the same experimental conditions (1, 3) However PR — an analog of the 3′ terminal end of aminoacyl-tRNA (21) — inhibits uridine uptake and decreases pre-rRNA synthesis, while it enhances the transcription of low-molecular weight RNA.

The ability of protein synthesis inhibitors to stimulate the synthesis of low-molecular weight RNA is a fact also observed in other biological systems (12, 13, 15, 23). Although it has been occasionally reported that PR may inhibit exogenous uridine incorporation into transfer (4 S) RNA when applied to ascites cells (4, 22), this result could be more a consequence of a non considered drug-produced decrease of the nucleoside uptake into the cells — as the present data suggest — than of a true drop of the synthesis of tRNA — as was interpreted (4, 22).

On the other hand, the enhancement of pre-rRNA synthesis when excised glands are treated with NC — or with some other inhibitors of translation (1, 3) — contrast with earlier observations. It has been reported in *Chironomus* larvae (8) and in other eukaryotic systems (6, 14, 16, 24) that transcription of the nucleolar genes is promptly decreased after cessation of protein synthesis. This different response of the transcriptional activity to inhibitors of translation is under investigation now (ALLER, in preparation).

Acknowledgements

This work has been partially supported by the «Comisión Asesora para Investigación Científica y Técnica», Spain. The author is greatly indebted to Dr. F.F. del Campo for the critical reading of the manuscript, to Mr. E. Wiltshire for checking the English translation, and to Dr. J. L. Díez for providing facilities at his Laboratory. Thanks are also given to Mrs. A. Partearroyo for drawing the graphs.

Resumen

Se estudia el efecto sobre la actividad de transcripción de dos inhibidores de la síntesis de proteínas, narciclasina y puromicina, cuando son aplicados por corto tiempo en glándulas salivales aisladas de *Chironomus thummi*. La narciclasina (6 μM) estimula la toma de uridina, así como la síntesis de ARN heterogéneo gigante, ARN prerribosómico (38 S) y ARN de bajo peso molecular (4-5 S). Por su parte, la puromicina (200 μM) inhibe la toma de uridina y deprime selectivamente la síntesis de ARN prerribosómico, en tanto que la síntesis de ARN de bajo peso molecular es ligeramente estimulada. Estos resultados se comparan con los ya conocidos como producidos por otros inhibidores de la traducción en este sistema biológico.

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