Modifications of Major Aspects of Myocardial Ribonucleic Acid Metabolism as a Response to Noradrenaline

ACTION OF THE HORMONE ON CYTOPLASMIC PROCESSING OF RIBONUCLEIC ACID AFTER RESERPINE TREATMENT

By ARNALDO CORTI,* AMOS CASTI,*† GABRIELE MEZZETTI,† NINO REALI,*
GIANCARLA ORLANDINI* and CLAUDIO M. CALDARERA†

*Istituto di Chimica Biologica dell'Università di Parma, Via Gramsci 14, 43100 Parma, Italy, and

†Istituto di Chimica Biologica dell'Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy

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Treatment of perfused rabbit heart with reserpine causes a decrease of incorporation of labelled precursors into RNA species of subcellular fractions and polyamines. Ornithine decarboxylase, *S*-adenosylmethionine decarboxylase and cytoplasmic Mn^{2+} -stimulated polyadenylate polymerase activities are not modified. Addition of noradrenaline to reserpine-treated perfused hearts enhances, compared with the control, the incorporation of precursor into RNA in all subcellular fractions other than the nuclear one, restores incorporation of labelled putrescine into polyamines, enhances ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities and causes a 12-fold increase in cytoplasmic Mn^{2+} -dependent polyadenylate polymerase activity. After treatment with noradrenaline the increase in radioactivity was found solely in AMP after hydrolysis of microsomal RNA to nucleoside monophosphates.

To obtain more convincing evidence supporting the suggestion that a post-transcriptional modification of RNA (namely a cytoplasmic polyadenylation) is one of the very first events after the injection of noradrenaline into perfused rabbit heart (see preceding paper, Casti *et al.*, 1977), the possibility of a relationship between the increase in incorporation of [3H]ribose into the RNA of the microsomal fraction and the stimulation of cytoplasmic Mn^{2+} -dependent polyadenylate polymerase activity elicited by noradrenaline has been studied more closely by using reserpine. This drug is known to cause the depletion of the stored endogenous catecholamines in the cardiac tissue (Bertler *et al.*, 1958; Alpers & Shore, 1969). Some effects of reserpine on nucleic acid metabolism have been described. The drug inhibits DNA synthesis in mouse liver (Hach *et al.*, 1972) and regenerating rat liver (Čihák & Vaptzarova, 1973).

Materials and Methods

All reagents and radioactive isotopes were as reported in the preceding paper (Casti *et al.*, 1977). The [3H]ribose used for the analysis of radioactivity of the ribonucleotides had a specific radioactivity of

9.6 Ci/mmol. Serpasil brand of reserpine was a gift from Ciba, Industria Chimica SpA, Milano, Italy.

The perfusion of hearts was carried out in every case for 40 min with the perfusion medium of Casti *et al.* (1977) to which reserpine (1 mg/15 ml of medium) was injected during the first 15 min of perfusion, at a rate of 1 ml/min, followed by perfusion medium containing the appropriate radioactive precursor. Noradrenaline (250 pmol/ml) was injected to reserpine-treated hearts, at a rate of 1 ml/min, at 2.5, 5 or 10 min before termination of perfusion.

Chromatographic separation of hydrolysates of RNA (Fleck & Munro, 1966) of microsomal fraction (Jones & Jones, 1969) was performed as described by Caldarera *et al.* (1962). The nucleotides of RNA plus corresponding internal standards were adsorbed on an ion-exchange resin column (1 cm × 25 cm) of Dowex 1 (X8; formate form; 200–400 mesh), and eluted by a formic acid/ammonium formate gradient. AMP, GMP, CMP and UMP were collected, determined by measuring A_{260} , dried and dissolved in 10 ml of scintillation 'cocktail' (Insta-Gel) for counting for radioactivity.

The methods used to determine the specific radioactivity of RNA and polyamines and the enzymic activities of ornithine decarboxylase, *S*-adenosylmethionine decarboxylase and Mn^{2+} -stimulated polyadenylate polymerase are described in the preceding paper (Casti *et al.*, 1977).

† To whom reprint requests should be addressed at Istituto di Chimica Biologica, Ospedale Maggiore, Via Gramsci 14, 43100 Parma, Italy.

Results

In Table 1 the response of [^3H]ribose incorporation into RNA of subcellular fractions to different doses of reserpine injected into perfused rabbit heart at a rate of 1 ml/min, is shown. All the doses tried (0.25, 0.50 or 1.00 mg/15 ml of perfused medium) were found to inhibit precursor incorporation into all RNA species, except mitochondrial RNA, which is unaffected by the lowest dose of the drug. Microsomal RNA is equally affected by all the three concentrations of reserpine, whereas for the other RNA species the most significant inhibition of specific radioactivity is obtained with a concentration of 1 mg/15 ml of perfusion medium.

Also [^{14}C]putrescine incorporation into spermidine and spermine is inhibited by reserpine injection into the heart (Table 2). The drug concentration giving a 50% decrease of specific radioactivity of both

polyamines is in the concentration range 0.50–1.00 mg/15 ml of perfusion medium.

Retention of labelled precursors in the hearts after treatment with reserpine (1 mg/15 ml) was tested at eight regular intervals during the 25 min of perfusion with radioactive isotopes, as described in the preceding paper (Casti *et al.*, 1977). The results showed a small decrease for [^3H]ribose retention (controls, 460 600 $\pm$ 21 600 d.p.m. per min of perfusion; reserpine-treated hearts, 358 700 $\pm$ 20 500 d.p.m. per min of perfusion) and 60% decrease of [^{14}C]putrescine retention (controls, 192 000 $\pm$ 8000 d.p.m. per min of perfusion; reserpine-treated hearts, 76 200 $\pm$ 4800 d.p.m. per min of perfusion).

The hearts treated with the higher concentration of reserpine (1 mg/15 ml) have also been used for determination of ornithine decarboxylase, S-adenosylmethionine decarboxylase and Mn^{2+} -stimulated polyadenylate polymerase activities *in vitro*. These

Table 1. *Effect of reserpine on specific radioactivity of RNA of subcellular fractions in perfused rabbit heart*

The perfusion rate was 10 ml/min. Various concentrations of reserpine were injected, at a rate of 1 ml/min, for 15 min; the perfusion of the hearts was then continued for 25 min with perfusion medium containing 50 μCi of [^3H]ribose/100 ml at the same perfusion rate. The technical details for separation of subcellular fractions and for RNA hydrolysis are reported in the preceding paper (Casti *et al.*, 1977). Results are expressed as means $\pm$ S.E.M.

Concn. of reserpine (mg/15 ml)	$10^{-3} \times$ Specific radioactivity (d.p.m./mg of RNA)			
	0	0.25	0.50	1.00
No. of expts.	8	6	6	6
Nuclei	2.90 $\pm$ 0.11	2.51 $\pm$ 0.10	1.70 $\pm$ 0.09	0.93 $\pm$ 0.06
Mitochondria	1.52 $\pm$ 0.08	1.50 $\pm$ 0.09	1.31 $\pm$ 0.08	0.70 $\pm$ 0.05
Microsomal fraction	3.10 $\pm$ 0.17	1.22 $\pm$ 0.08	1.11 $\pm$ 0.07	1.01 $\pm$ 0.06
Postmicrosomal fraction	2.73 $\pm$ 0.12	2.02 $\pm$ 0.11	1.62 $\pm$ 0.10	1.20 $\pm$ 0.08

Table 2. *Effect of reserpine on specific radioactivity of polyamines in perfused rabbit heart*

The perfusion rate was 10 ml/min. Various concentrations of reserpine were injected, at a rate of 1 ml/min, for 15 min; the perfusion of the hearts was then continued for 25 min with perfusion medium containing 5 μCi of [^{14}C]putrescine/100 ml at the same perfusion rate. The details of extraction and separation of polyamines are reported in the preceding paper (Casti *et al.*, 1977). Results are expressed as means $\pm$ S.E.M. for six experiments.

Concn. of reserpine (mg/15 ml)	Specific radioactivity (d.p.m./nmol of polyamine)			
	0	0.25	0.50	1.00
Spermidine	125.3 $\pm$ 6.9	107.5 $\pm$ 8.0	69.1 $\pm$ 5.1	60.5 $\pm$ 4.3
Spermine	12.5 $\pm$ 0.7	10.4 $\pm$ 0.8	6.6 $\pm$ 0.5	6.3 $\pm$ 0.4

Table 3. *Effect of reserpine on ornithine decarboxylase, S-adenosylmethionine decarboxylase and cytoplasmic Mn^{2+} -stimulated polyadenylate polymerase activities in perfused rabbit heart*

The perfusion rate was 10 ml/min. Reserpine (1 mg) was injected, at a rate of 1 ml/min, for 15 min; the perfusion of the hearts was then continued for 25 min with perfusion medium. The assays of enzymic activities are reported in the preceding paper (Casti *et al.*, 1977). Results are expressed as means $\pm$ S.E.M. for six experiments.

	Ornithine decarboxylase (pmol of $^{14}\text{CO}_2$ /60 min per mg of protein)	S-Adenosylmethionine decarboxylase (pmol of $^{14}\text{CO}_2$ /30 min per mg of protein)	Polyadenylate polymerase (nmol of AMP incorporated/ 30 min per mg of protein)
Control	10.2 $\pm$ 0.4	19.5 $\pm$ 1.1	1.20 $\pm$ 0.04
Reserpine-treated	11.0 $\pm$ 0.7	20.1 $\pm$ 1.6	1.01 $\pm$ 0.05

activities, all of which were stimulated by noradrenaline (Casti *et al.*, 1977), are unaffected by the addition of the drug to the organs (Table 3).

Fig. 1 reports the effect of noradrenaline on precursor incorporation into RNA of subcellular fractions of reserpine-treated perfused heart. The various RNA species behave differently after the treatment with the hormone. In the mitochondrial, microsomal and postmicrosomal fractions the strong decrease of RNA-specific radioactivity caused by injection of reserpine is overcome 2.5 min after addition of 250 pmol of adrenaline/ml per min.

Fig. 1. Effect of noradrenaline on incorporation of [³H]ribose into RNA of subcellular fractions of reserpine-treated perfused rabbit heart

The perfusion rate was 10 ml/min. Reserpine (1 mg/15 ml of perfusion medium) was injected, at a rate of 1 ml/min, for 15 min; the perfusion was then continued for 25 min with perfusion medium containing 50 μCi of [³H]ribose/100 ml. Noradrenaline (250 pmol/ml per min) was injected 2.5, 5 or 10 min before termination of perfusion. The technical details for separation of subcellular fractions and for RNA hydrolysis are reported in the preceding paper (Casti *et al.*, 1977). Each point represents the results for six animals; the S.E.M. was <10%. ○, Nuclei; ■, mitochondria; ▲, microsomes; ●, postmicrosomal fraction.

The specific radioactivity of mitochondrial and microsomal RNA continues to rise steeply up to 5 min, reaching respectively 100% and 400% of the control. At 10 min the specific radioactivity of RNA of the mitochondrial fraction falls back to the control value, whereas that of the microsomal fraction is still increased by 80%. The incorporation of label into postmicrosomal RNA remains approximately the same as control values up to 10 min after hormone injection. In contrast with this behaviour, the specific radioactivity of nuclear RNA is not influenced by noradrenaline treatment and stays inhibited at all the times studied.

Also the inhibition of [¹⁴C]putrescine incorporation into spermidine and spermine caused by injection of reserpine into the heart is overcome by administration of noradrenaline (Table 4). The restoration by the hormone of the specific radioactivity of polyamines, however, is delayed compared with that of ribonucleic acids, and no stimulatory effect comparable with that obtained in the absence of reserpine

Fig. 2. Stimulation of ornithine decarboxylase (a) and S-adenosylmethionine decarboxylase (b) activities by noradrenaline in reserpine-treated perfused rabbit heart

The perfusion rate was 10 ml/min. Reserpine (1 mg/15 ml of perfusion medium) was injected, at a rate of 1 ml/min, for 15 min; the perfusion was then continued for 25 min with perfusion medium. Noradrenaline (250 pmol/ml per min) was injected 2.5, 5 or 10 min before ending of perfusion. The assays of enzymic activities are reported in the preceding paper (Casti *et al.*, 1977). Each point represents the results for six animals; the S.E.M. was <10%.

Table 4. Effect of noradrenaline on specific radioactivity of polyamines in reserpine-treated perfused rabbit heart The perfusion of the hearts was as described in the legend of Fig. 1, except that [¹⁴C]putrescine (5 μCi/100 ml) was the labelled precursor used. The details for extraction and separation of polyamines are reported in the preceding paper (Casti *et al.*, 1977). Results are expressed as means ± S.E.M. for six experiments.

		Specific radioactivity (d.p.m./nmol of polyamine)			
		Time period of noradrenaline injection into reserpine-treated hearts (min)			
	Control	Reserpine (1 mg/15 ml)	2.5	5.0	10.0
Spermidine	125.3 ± 6.9	60.5 ± 4.3	69.1 ± 5.1	125.0 ± 7.1	127.1 ± 7.0
Spermine	12.5 ± 0.8	6.3 ± 0.4	6.2 ± 0.4	10.5 ± 0.8	10.5 ± 0.8

treatment (see the preceding paper, Casti *et al.*, 1977) is found with noradrenaline within 10 min.

The measurement of radioactivity retained by reserpine-treated hearts under the action of noradrenaline indicates that the hormone immediately causes, as in non-reserpine-treated organs (see Casti *et al.*, 1977), an increase that is twice the control value for [^3H]ribose and reaches the control value for [^{14}C]putrescine, these values being maintained throughout the time of hormone treatment (results not shown).

Ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities, which are not influenced by injection of reserpine into hearts, are differently stimulated by exogenous noradrenaline added to the reserpine-treated heart (Fig. 2). Both decarboxylases are unmodified at 2.5 min of hormone treatment;

Fig. 3. Stimulation of cytoplasmic Mn^{2+} -stimulated polyadenylate polymerase activity by noradrenaline in reserpine-treated perfused rabbit heart

The perfusion rate was 10 ml/min. Reserpine (1 mg/15 ml of perfusion medium) was injected, at a rate of 1 ml/min, for 15 min; the perfusion was then continued for 25 min with perfusion medium. Noradrenaline (250 pmol/ml per min) was injected 2.5, 5 or 10 min before termination of perfusion. The assay of enzymic activity is reported in the preceding paper (Casti *et al.*, 1977). Each point represents the results for six animals; the S.E.M. was <10%. ■, Control; ○, noradrenaline-treated.

at 5 min ornithine decarboxylase activity is enhanced 2-fold and *S*-adenosylmethionine decarboxylase activity only by 25%; and at 10 min the former activity is still 50% stimulated and the latter falls to near the control value.

Fig. 3 shows that also in reserpine-treated hearts, as in non-treated organs (Casti *et al.*, 1977), a huge enhancement of cytoplasmic Mn^{2+} -stimulated polyadenylate polymerase activity occurs after injection of noradrenaline: 5 min of treatment give the highest stimulation, which is 12-fold over the control. At 10 min the enzymic activity is still strongly stimulated.

To study the distribution of radioactivity among ribonucleotide monophosphates obtained on hydrolysis of microsomal RNA, the reserpine-treated hearts were perfused with perfusion medium containing [^3H]ribose and treated for 5 min with noradrenaline. Radioactivity is evenly distributed among the four ribonucleoside monophosphates in non-treated hearts, whereas in reserpine- and hormone-treated organs radioactivity is enhanced severalfold solely in AMP (Table 5).

Discussion

It appeared from the preceding paper (Casti *et al.*, 1977) that changes in specific radioactivity of RNA induced by injection of noradrenaline into perfused rabbit heart do not imply modifications in the mechanisms of synthesis of these macromolecules *de novo*. The results suggested rather that post-transcriptional processes required for RNA metabolism and function are involved. More unequivocal support for that idea comes from the present paper, where reserpine-treated hearts were used.

The decrease in specific radioactivity of polyamines caused by reserpine can be accounted for by the diminished retention of [^{14}C]putrescine within the organs under the effect of this drug. The enzymes involved in the first two steps of polyamine synthesis are in fact unmodified in the reserpine-treated hearts (Table 3).

When noradrenaline is administered to the reserpine-treated organs, [^{14}C]putrescine retention in them is restored to the control value, but no enhancement occurs comparable with that obtained when the hormone was given to non-reserpine-treated organs (see the preceding paper, Casti *et al.*, 1977). The same

Table 5. Distribution of [^3H]ribose between the nucleoside monophosphates from hydrolysates of microsomal RNA [^3H]Ribose (500 $\mu\text{Ci}/100\text{ml}$) was used as RNA precursor. This experiment was carried out with reserpine-treated (1 mg/15 ml) hearts injected with noradrenaline (250 pmol/ml per min) for 5 min. Results are expressed as means $\pm$ S.E.M. for six experiments.

	Radioactivity (d.p.m.)			
	CMP	AMP	GMP	UMP
Control	101 $\pm$ 6	152 $\pm$ 9	138 $\pm$ 8	132 $\pm$ 9
Noradrenaline-treated	160 $\pm$ 9	1602 $\pm$ 95	210 $\pm$ 15	160 $\pm$ 10

pattern is followed by the incorporation of [^{14}C]putrescine into both spermidine and spermine (Table 4). The effect of noradrenaline on the first two enzymes of polyamine synthesis is still manifest under the effect of reserpine (Fig. 2), but, whereas ornithine decarboxylase activity is stimulated to the same extent as in non-reserpine-treated organs [see Fig. 2 of the preceding paper (Casti *et al.*, 1977)], the increase of *S*-adenosylmethionine decarboxylase activity in reserpine-treated heart is probably too small to be reflected in a detectable increase of specific radioactivity of polyamines (Table 4).

Retention of [^3H]ribose within the heart is less influenced by reserpine than is that of [^{14}C]putrescine, and it cannot totally account for the decrease of RNA specific radioactivity. Since polyadenylate polymerase is not modified in reserpine-treated heart, it is conceivable that the drug has some inhibitory effect on another metabolic process. Reserpine is an uncoupling agent of oxidative phosphorylation in mitochondria of kidney and liver of monkeys (Maina, 1974). Should this also be the case for rabbit heart, a decreased availability of ATP would represent a further explanation for decreased incorporation of label into ribonucleic acids. This hypothesis requires, however, that the putative inhibitory effect on ATP production be overcome by subsequent injection of noradrenaline. The latter is able to enhance the specific radioactivity in the microsomal fraction to a much higher extent than expected if it was exclusively due to a noradrenaline-dependent increase of [^3H]ribose retention. An increased nucleotide triphosphate incorporation must occur under these circumstances for which a restored availability of ATP is obligatory. The stimulatory effect of the hormone on glycolysis (Mayer, 1974) makes this not at all inconceivable. The strongly augmented specific radioactivity of microsomal RNA appears therefore more clearly linked to the dramatic enhancement of cytoplasmic Mn^{2+} -dependent polyadenylate polymerase activity that occurs concomitantly. It is in fact easier to distinguish from that of the other RNA species (Fig. 1) than it is in the absence of the drug [Table 1 of the preceding paper (Casti *et al.*, 1977)]. In particular,

the specific radioactivity of nuclear RNA remains less than the control values even in the presence of noradrenaline (possibly owing to interference of the drug with the genetic apparatus), strengthening the conclusion that the metabolism of RNA is influenced by noradrenaline at a cytoplasmic level.

The analysis of radioactivity of ribonucleoside monophosphates resulting from hydrolysis of RNA of microsomal fractions represents, in our opinion, a rather conclusive way to decide whether the increase of incorporation of label into this RNA under the action of noradrenaline in reserpine-treated heart is in fact attributed to stimulation of synthesis of RNA or to its activation in the cytoplasm. For the microsomal fraction, the increase of radioactivity exclusively in AMP demonstrates that indeed this can be due to stimulation of polyadenylation of mRNA molecules in the cytoplasm under the action of noradrenaline.

In conclusion, results from the present and the preceding paper (Casti *et al.*, 1977) suggest that it is not a nuclear transcriptional modification but a cytoplasmic processing of RNA that is triggered as a rapid response of the heart to noradrenaline treatment.

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