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

BEHAVIOUR OF POLYADENYLATE POLYMERASE AND RIBONUCLEIC ACID POLYMERASE, ACETYLATION OF HISTONES AND RATE OF SYNTHESIS OF POLYAMINES

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Noradrenaline added to perfused rabbit heart previously perfused with labelled precursors causes, after 2.5 and 5.0 min, a general increase of specific radioactivity of RNA in sub-cellular fractions, but no augmentation of acetylation of F2a2 and F2a1 histone fractions and no stimulation of DNA-dependent RNA polymerase activities. Synthesis of spermidine and spermine is enhanced at 10.0 min of treatment, when there is also a fall in specific radioactivity of RNA. The cytoplasmic Mn^{2+} -stimulated polyadenylate polymerase activity is strongly enhanced 30 s to 2.5 min after injection of noradrenaline or of dibutyryl cyclic AMP. Both the cyclic nucleotide and noradrenaline have no influence *in vitro* on the polyadenylate polymerase reaction.

It has been widely recognized that catecholamines increase the rate of mobilization and utilization of energy sources within the heart (Williams & Mayer, 1966; Wollenberger *et al.*, 1969; Mayer, 1974). Owing to their stimulatory effect on the force and frequency of contraction, the demand for an adequate supply of enzymic and contractile proteins is also conceivably increased under their action (Mayer, 1974). This in turn should reflect changes at the level of nucleic acid metabolism. Evidence exists for a stimulatory action by catecholamines on precursor incorporation into proteins (Mallov, 1973, 1976; Kallfelt *et al.*, 1976), RNA and polyamines (Decalonne *et al.*, 1967; Caldarera *et al.*, 1970, 1971).

In the present paper we have studied the effect of noradrenaline on several processes all involved in the transcriptional and translational mechanisms of RNA in the perfused rabbit heart.

ATP (disodium salt), GTP (trisodium salt), CTP (disodium salt), UTP (tetrasodium salt) by Sigma Chemical Co., St. Louis, MO, U.S.A., and L-ornithine and pyridoxal 5-phosphate by Merck, Darmstadt, West Germany. α -Amanitin was from Boehringer und Soehne, Ingelheim, West Germany, poly(A) from Miles Laboratories Inc., Kankakee, IL, U.S.A., and Insta-Gel scintillation fluid from Packard Instrument Co., La Grange, IL, U.S.A. [3H]-Ribose (2.9 Ci/mmol), [^{14}C]putrescine (54 mCi/mmol) and sodium [^{14}C]acetate (59 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks, U.K. DL-[^{14}C]Ornithine monohydrochloride (42 mCi/mmol), S-adenosyl-L-[^{14}C]methionine (54 mCi/mmol), [8- ^{14}C]adenosine 5'-triphosphate (tetrasodium salt) (51 mCi/mmol) and [5,6- 3H]uridine 5'-triphosphate (tetrasodium salt) (40 Ci/mmol) were from New England Nuclear G.m.b.H., Dreieichenhain, West Germany.

Experimental

Materials

Noradrenaline hydrochloride and 1,4-diaminobutane dihydrochloride were supplied by Fluka A.G., Buchs, Switzerland, acrylamide, *NN'*-bisacrylamide and *NNN'*-tetramethylethylenediamine by Bio-Rad Laboratories, Richmond, CA, U.S.A., *N*⁶*O*^{2'}-dibutyryl cyclic AMP, S-adenosyl-L-methionine,

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Perfusion of hearts

Male and female 3-month-old rabbits weighing about 2 kg from a commercial source were used in all experiments. The perfusion of the hearts was performed without recirculation (drip-through) retrogradely through the aorta in a Langendorff (1895) apparatus. The composition of the perfusion medium was: 154 mM-NaCl, 5.6 mM-KCl, 2.2 mM-CaCl₂, 5.9 mM-NaHCO₃ and 5.5 mM-glucose. The flow rate of the perfusion medium was 10 ml/min. The radioactive precursors were added to the medium at the

beginning of perfusion so that the macromolecules could become sufficiently labelled. The perfusion of the hearts was carried out for 25 min, and noradrenaline or *N*⁶*O*^{2'}-dibutyryl cyclic AMP dissolved in the perfusion medium was injected by a pump into the hearts, at a rate of 1 ml/min, 30s, 1, 2.5, 5 or 10 min before termination of perfusion. The experiments were terminated by freezing the hearts in a Wollenberger clamp cooled in liquid N₂, while the hearts were still being perfused; the left ventricle was separated and used for several determinations. For separation of subcellular fractions taken for RNA extraction, fresh hearts were used.

Isolation of nuclei

The isolation of nuclei for extraction of histones was based on the method of Hnilica *et al.* (1966). The rabbit hearts were homogenized in 0.25 M-sucrose containing 2 mM-CaCl₂ and 2 mM-MgCl₂ in an Ultra-Turrax homogenizer. The homogenate was strained through six layers of cheesecloth, then layered over an equal volume of 0.34 M-sucrose containing 2 mM-MgCl₂ and centrifuged at 1000g for 20 min. The pellet was suspended in 0.25 M-sucrose containing 1 mM-CaCl₂ and 1 mM-MgCl₂, and the suspension layered over 0.34 M-sucrose alone and centrifuged at 1000g for 15 min. The nuclei were purified by resuspending the pellet in 2.2 M-sucrose and centrifuging at 105000g for 60 min.

The nuclei used for assaying RNA polymerases were prepared as indicated by Liao *et al.* (1968). The left ventricles were homogenized with 2.2 M-sucrose containing 1 mM-MgCl₂, adjusted to 50 ml with the same medium and layered on 10 ml of the same hyperosmotic sucrose. After centrifuging for 60 min at 20000 rev./min in a Spinco SW 25.2 rotor, the nuclear pellet was resuspended with 0.25 M-sucrose.

Separation of subcellular fractions

Subcellular fractions were separated as described by Jones & Jones (1969). The left ventricles were homogenized in 8 vol. of medium containing (final concentrations) 0.25 M-sucrose, 4 mM-Tris/HCl, pH 7.2, and 1 mM-EDTA. After sedimentation of the nuclear fraction by centrifugation at 800g for 10 min, the supernatant was centrifuged at 17000g for 4 min and the mitochondrial pellet was washed twice in the same buffer. The combined washings and supernatants were centrifuged at 15000g for 15 min, and the sediment was added to the previous mitochondrial pellet. The ribosomes were sedimented from the supernatant by centrifugation at 105000g for 45 min. The postmicrosomal fraction was precipitated with 0.5 M-HClO₄ (final concentration) and centrifuged at 10000g for 10 min.

Analytical methods

[5-³H]Ribose (50 μCi/100 ml of perfusion medium) was used as precursor of RNA. The RNA of subcellular fractions was determined by the method of Fleck & Munro (1966). The hydrolysed RNA was neutralized, measured by the *A*₂₆₀ and then dried. Finally, 10 ml of a scintillation 'cocktail' (Insta-Gel) was added and the radioactivity was measured in a Packard Tri-Carb liquid-scintillation spectrometer.

The two DNA-dependent RNA polymerase activities were assayed by the method of Novello & Stirpe (1970). Purified nuclei (60–80 μg of DNA) were incubated for 15 min in the following reaction mixtures (final vol., 0.6 ml): for nucleolar RNA polymerase, 50 μmol of Tris/HCl buffer, pH 8.0, 2 μmol of MgCl₂, 2 μmol of 2-mercaptoethanol, 3 μmol of NaF, 0.3 μmol each of ATP, GTP and CTP, 0.015 μmol of [³H]UTP (293 d.p.m./pmol), and 1 μg of α-amanitin; for nucleoplasmic RNA polymerase, 50 μmol of Tris/HCl buffer, pH 8.0, 2 μmol of MnCl₂, 0.14 mmol of (NH₄)₂SO₄, 0.9 μmol each of ATP, GTP and CTP, 0.045 μmol of [³H]UTP (98 d.p.m./pmol) and 1 μg of α-amanitin (when present). The reactions were stopped by adding 1 mg of serum albumin and 5 ml of 0.5 M-HClO₄ at 0°C.

Sodium [1-¹⁴C]acetate (25 μCi/100 ml of perfusion medium) was used for acetylation of histone fractions in perfused hearts. The histones were extracted from purified nuclei as follows (Hnilica *et al.*, 1966). The nuclear pellet was suspended in 0.14 M-NaCl containing 0.01 M-trisodium citrate, pH 7.2, and centrifuged at 1600g for 20 min. The sediment was washed in 0.1 M-Tris/HCl, pH 7.6, and centrifuged at 1600g for 15 min. The resulting sediment was extracted with 95% (v/v) ethanol, centrifuged at 1600g for 15 min and the histones were extracted with 0.2 M-HCl. The histone fractions were precipitated by adding 8 vol. of acetone, washed twice and dried under vacuum. All the operations were performed at 0–4°C. Analytical gel electrophoresis was performed, as described by Panyim & Chalkley (1969), with 0.9 M-acetic acid at 2 mA/7.5 cm of gel at 80–120 V. Histones (1 mg/ml) were dissolved in 0.9 M-acetic acid in 15% (w/v) sucrose. The amount of solution applied to the gels was 50 μl. The gels were scanned at 280 nm by an ISCO model UA-4 absorbance monitor with an ISCO model 658 gel-scanner transport attachment. The histone content was calculated by integration of peak areas; histone fraction (20 μg) of calf thymus, extracted and purified by the method of Johns (1964), was used as protein standard. For radioactivity measurements, the gels were stained with 0.1% (w/v) Amido Black in ethanol/acetic acid (20:7, v/v) and destained with 0.9 M-acetic acid. The coloured bands were cut out, dissolved in 2 M-NaOH and added to 10 ml of scintillation 'cocktail' (Insta-Gel).

The polyamines were determined by the method of

Raina & Cohen (1966). [1,4-¹⁴C]Putrescine (5 μ Ci/100ml) was added to the perfusion medium at the beginning of perfusion. The left ventricles were homogenized in a glass Potter homogenizer in 3% (w/v) HClO₄. The supernatants were neutralized with 4M-KOH and the KClO₄ was removed by centrifugation at 10000g for 15min. The spermidine and spermine were extracted with butanol; the extracts were evaporated to dryness and then dissolved in 0.1M-HCl. The polyamines were separated by electrophoresis at 9V/cm on Whatman no. 1 paper for 3h in 0.1M-sodium citrate buffer, pH3.5. The spermidine and spermine, stained with ninhydrin, were eluted with water/acetic acid/ethanol (1:5:4, by vol.) containing 2% (w/v) cadmium acetate, and then determined by *A*₅₀₅. For radioactivity measurements, the coloured bands were cut out and placed in counting vials with 10ml of scintillation 'cocktail' (Insta-Gel).

Ornithine decarboxylase activity was determined by the method of Jänne & Williams-Ashman (1971). The incubation mixture contained, in a final volume of 1.0ml, 100mM-glycylglycine buffer, pH7.2, 0.25mM-L-ornithine, 1 μ Ci of DL-[¹⁴C]ornithine, 5mM-dithiothreitol, 0.2mM-pyridoxal 5-phosphate and approx. 10mg of protein. The incubation was carried out for 60min at 37°C.

The enzymic decarboxylation of *S*-adenosyl-L-[carboxy-¹⁴C]methionine was determined by the method of Pegg & Williams-Ashman (1969). The reaction mixtures contained 100mM-sodium phosphate buffer, pH7.2, 0.2mM-*S*-adenosyl-L-[carboxy-¹⁴C]methionine (10.99d.p.m./pmol), 10mM-dithiothreitol, 2.5mM-putrescine and enzyme (5–6mg of protein) in a final volume of 1.0ml. The tubes were incubated for 30min at 37°C. All reactions were initiated by the addition of the appropriate enzyme and stopped by adding 1ml of trichloroacetic acid (40%, w/v). Crude extracts of the perfused hearts for the two decarboxylase assays were prepared by homogenization with 3 vol. of 10mM-sodium phos-

phate buffer, pH7.2, containing 2mM-dithiothreitol and 0.1mM-EDTA, followed by centrifugation at 35000g for 30min at 2°C.

The enzymic extract for cytoplasmic Mn²⁺-dependent polyadenylate polymerase (polynucleotide adenyltransferase, EC 2.7.7.19) was the supernatant (105000g, 60min) of a homogenate made in ice-cold 0.25M-sucrose containing 50mM-Tris/HCl (pH7.5), 25mM-KCl and 5mM-MgCl₂, from which nuclei and mitochondria had been removed by centrifugation (Tsiapalis *et al.*, 1975). The standard test system (Corti *et al.*, 1975) contained 100mM-Tris/HCl, pH8.2 (37°C), 2mM-dithiothreitol, 1mg of poly-(A)/ml, 1mM-[8-¹⁴C]ATP (1200–1500 d.p.m./nmol), 1mM-MnCl₂ and approx. 0.3mg of protein, with water to a final volume of 0.25ml. After 30min at 37°C, the reactions were terminated by addition of ice-cold trichloroacetic acid to a final concentration of 5%. The washed acid-insoluble precipitate was dissolved in 88% (v/v) formic acid and its radioactivity was determined by scintillation counting (Calvin *et al.*, 1967).

The concentration of proteins was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

The action of the most effective concentrations (250 or 500pmol/ml, at a rate of 1ml/min) of noradrenaline added to the perfused rabbit heart on the specific radioactivities of RNA species of subcellular fractions is shown in Table 1. The pattern of enhancement of specific radioactivity has been studied within 10min of noradrenaline treatment. The incorporation of [³H]ribose into all RNA species rapidly increases 2.5min after hormone injection, reaching a peak at 5min and falling back at 10min, possibly indicating a time effect of the hormone on precursor incorporation, as also observed by Decalonne *et al.* (1967) and Caldarera *et al.* (1970).

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

The perfusion rate was 10ml/min. The heart was perfused for 25min with perfusion medium containing [5-³H]ribose. Noradrenaline solutions were injected, at 1ml/min, 2.5, 5 or 10min before termination of perfusion. The details for separation of subcellular fractions and for RNA hydrolysis are reported in the Experimental section. Each point represents results for six animals; the S.E.M. was less than 10%. Control values are expressed as 10⁻³ × specific radioactivity (d.p.m./mg of RNA); others are expressed as percentages of the control (control = 100%).

Time period of injection of noradrenaline (min ... 0		Specific radioactivity (% of control)					
		2.5		5.0		10.0	
		250	500	250	500	250	500
Noradrenaline added (pmol/ml per min) ...	(Control)						
Nuclei	2.90 ± 0.11	268	200	310	252	154	125
Mitochondria	1.52 ± 0.08	275	270	350	290	135	212
Microsomal fraction	3.10 ± 0.17	254	163	410	238	190	115
Postmicrosomal fraction	2.73 ± 0.12	290	158	385	213	290	100

The most pronounced effect is obtained with 250 pmol/ml injected at a rate of 1 ml/min in all cases, except for the mitochondrial RNA at 10 min.

When the action of the hormone on precursor uptake was considered by measuring the radioactivity of the perfusion fluid leaving the organ, it was shown that noradrenaline causes, immediately after injection and throughout the time of treatment, an increase in the radioactivity retained by the organ. (Radioactivity was measured in the perfusate of non-treated organs. An average of eight samples were collected at regular time intervals and the results are means of six hearts. In the perfusate from hearts after injection of noradrenaline, an average of ten samples were collected at regular time intervals and the results are means of six hearts. The radioactivity retained was: $460\,600 \pm 21\,600$ d.p.m. per min of perfusion in non-treated organs, and $1185\,900 \pm 54\,600$ d.p.m. per min of perfusion after noradrenaline injection. Values are significantly different from

controls, $P < 0.01$). Under different experimental conditions a facilitated entry of precursor by catecholamines has been shown also by Isaksson & Kallfelt (1972).

In Fig. 1 the effect of noradrenaline (250 pmol/ml at a rate of 1 ml/min for 2.5, 5 and 10 min) on incorporation of [14 C]acetate into histone fractions of perfused heart is shown. A general decrease of acetylation occurs in histones F2a2 and F2a1 at all the times considered, whereas in the other fractions incorporation is either practically unaffected or slightly inhibited, except for histone F1 at 2.5 min and histone F2b at 5 min, where it is enhanced.

The polymerization reactions of RNA occurring within the nuclei (Table 2) show a slight decrease after 2.5 min of hormone treatment. Longer times cause a rise in the activities of the two polymerases, more evident for the nucleoplasmic enzyme, at 10 min.

The influence of noradrenaline on [14 C]putrescine incorporation into spermidine and spermine has also been studied (Table 3). The doses of hormone and times of treatment are the same as those for studying the specific radioactivity of RNA. The hormone causes an increase of specific radioactivity in both polyamines, the most effective dose being again 250 pmol/ml per min. The time effect is not the same as with RNA. Incorporation increases slightly at 2.5 min, continues to rise at 5 min and is 3–4-fold greater than the control values at 10 min after noradrenaline injection. Also, as for the results for [3 H]ribose incorporation, the hormone causes an increase (100%) of precursor uptake into the organ at all times immediately after injection (results not shown). The most effective dose of hormone has been used to study the enzyme activities that are involved in spermidine and spermine synthesis (Fig. 2). As expected from the previous data, both enzymic activities are stimulated by noradrenaline. The peak of rate enhancement is at 2.5 min for ornithine decarboxylase and 5 min for *S*-adenosylmethionine decarboxylase activity.

The dramatic modifications of Mn^{2+} -dependent polyadenylate polymerase activity, mainly cytoplasmic (Tsiapalis *et al.*, 1975), after hormone injection into the perfused heart, are shown in Fig. 3. That a polyadenylation reaction is always measured is demonstrated by the complete dependence of AMP incorporation on the addition of synthetic poly(A) to the reaction mixture (results not shown). A 6-fold increase of AMP incorporation into acid-precipitable material occurs after only 30 s of noradrenaline treatment, and the peak of stimulation is reached after 1 min. At 5 min the polyadenylate polymerase activity is still 4-fold greater than the control values but at 10 min the stimulation has ceased.

Cyclic AMP is known to be the mediator of most of the metabolic actions of noradrenaline. Injection of

Fig. 1. Effect of noradrenaline on acetylation of histone fractions in perfused rabbit heart

The hearts were perfused with perfusion medium containing sodium [14 C]acetate, at a rate of 10 ml/min. Noradrenaline (250 pmol/ml) was injected, at a rate of 1 ml/min, 2.5, 5 or 10 min before termination of perfusion. The control values (six determinations), expressed as d.p.m./ μ g of protein, are: F1 120 ± 9 ; F3 168 ± 15 ; F2b 101 ± 8 ; F2a2 154 ± 11 ; F2a1 233 ± 19 . In all other cases, the results are the means of the results for six hearts and the S.E.M. was $<10\%$. For technical details see the Experimental section. Values are significantly different from controls ($P < 0.01$) in the following cases: histones F2a2 and F2a1 throughout all the time of treatment; F1 at 2.5 min and F2b at 5 min. $\circ$, F1; Δ , F3; $\blacksquare$, F2b; $\bullet$, F2a2; $\blacktriangle$, F2a1.

Table 2. *Effect of noradrenaline on DNA-dependent RNA polymerase activities in perfused rabbit heart*
The perfusion rate was 10ml/min. The perfusion time was 25 min; at 2.5, 5 or 10 min before termination of perfusion, noradrenaline (250 pmol/ml) was injected at a rate of 1 ml/min. Data represent the means of six hearts (three samples per determination) $\pm$ s.e.m. For the assay of enzymic activities, see the Experimental section.

Time period of injection of noradrenaline (min) ...	RNA polymerase activity (pmol of [³ H]UMP incorporated/15 min per mg of DNA)			
	0	2.5	5.0	10.0
RNA polymerase				
Nucleolar	70 $\pm$ 5	68 $\pm$ 6	90 $\pm$ 8	88 $\pm$ 8
Nucleoplasmic	210 $\pm$ 15	170 $\pm$ 14	220 $\pm$ 18	300 $\pm$ 26

Table 3. *Effect of noradrenaline on polyamine specific radioactivity in perfused rabbit heart*
The hearts were perfused, at a rate of 10ml/min, with perfusion medium containing [1,4-¹⁴C]putrescine throughout the period of the experiment (25 min). Noradrenaline solutions, at a rate of 1 ml/min, were added 2.5, 5 or 10 min before termination of perfusion. The technical details are reported in the Experimental section. Each point represents the results for six animals; the s.e.m. was <10%. Control values are expressed as specific radioactivities (d.p.m./nmol of polyamine); others are expressed as percentages of the control (control = 100%).

Time period of injection of noradrenaline (min) ...	0	Specific radioactivity (% of control)					
		2.5		5.0		10.0	
Noradrenaline added (pmol/ml per min) ...	(Control)	250	500	250	500	250	500
Spermidine	125.3 $\pm$ 6.9	125	115	150	130	313	200
Spermine	12.5 $\pm$ 0.8	113	105	125	110	380	310

Fig. 2. *Ornithine decarboxylase and S-adenosylmethionine decarboxylase activities in perfused rabbit heart after treatment with noradrenaline*

The hearts were perfused, at a rate of 10ml/min, for 25 min, and noradrenaline (250 pmol/ml) was injected, at a rate of 1 ml/min, 2.5, 5 or 10 min before termination of perfusion. Data represent the means of results for six hearts (three samples per determination) and the s.e.m. was <10%. For the assay of enzymic activities, see the Experimental section. (a) Ornithine decarboxylase; (b) S-adenosylmethionine decarboxylase.

Fig. 3. Effect of noradrenaline on the pattern of cytoplasmic Mn^{2+} -stimulated polyadenylate polymerase activity of perfused rabbit heart

The hearts were perfused, at a rate of 10 ml/min, for 25 min and noradrenaline (250 pmol/ml) was injected at a rate of 1 ml/min, at 30 s, 1, 2.5, 5 or 10 min before termination of perfusion. Data represent the means of the results for six hearts (three samples per determination) and the s.e.m. was <10%. The assay of enzyme is reported in the Experimental section.

Fig. 5. Time course of the effect of dibutyryl cyclic AMP on cytoplasmic Mn^{2+} -stimulated polyadenylate polymerase activity in perfused rabbit heart

The hearts were perfused, at a rate of 10 ml/min, for 25 min, and the dibutyryl cyclic AMP (0.1 mM) was injected, at a rate of 1 ml/min, 2.5, 5 or 10 min before termination of perfusion. Data represent the means of the results for six hearts (three samples per determination) and the s.e.m. was <10%. The assay of enzymic activity is reported in the Experimental section.

Fig. 4. Effect of dibutyryl cyclic AMP on cytoplasmic Mn^{2+} -dependent polyadenylate polymerase activity of perfused rabbit heart

The hearts were perfused, at a rate of 10 ml/min, for 25 min. At 2.5 min before termination of perfusion, the indicated concentrations of dibutyryl cyclic AMP were injected at a rate of 1 ml/min. Data represent the means of the results for six hearts (three samples per determination) and the s.e.m. was <10%. The enzyme assay is reported in the Experimental section.

dibutyryl cyclic AMP into the perfused rabbit heart resulted in a strong stimulation of soluble polyadenylate polymerase. The most effective dose was in the range 10–100 μM (Fig. 4). Stimulation by 100 μM -

cyclic AMP occurred after 2.5 min, and did not significantly decrease at 5 and 10 min (Fig. 5).

Dibutyryl cyclic AMP injected into the heart at a concentration of 100 μM for 2.5, 5 and 10 min did not increase the activity of RNA polymerase of the nuclei (results not shown).

Noradrenaline at concentrations ranging from 0.4 nM to 40 μM and cyclic AMP in the range 0.2 μM to 0.2 mM had no effect when added to the assay system for soluble polyadenylate polymerase (results not shown).

Discussion

The enhancement of specific radioactivity of RNA by noradrenaline under our experimental conditions (i.e. a system *in vitro*, where all compensatory homeostatic mechanisms are excluded) can only be accounted for partially by the increased uptake of labelled precursor. The possible action of noradrenaline on processes related to induction of RNA synthesis has therefore been considered.

Histone fractions F2a2 and F2a1 are acetylated in the 'internal' lysine residues and this kind of acetylation appears to be responsible for RNA synthesis (Pogo *et al.*, 1968; Allfrey, 1971). The decrease of acetylation of these fractions under our experimental

conditions suggests that this mechanism of gene de-repression is not activated. For lysine-rich histone fractions, it has been suggested that the acetylation occurs in the cytoplasm and is connected with histone synthesis (Allfrey, 1971).

In the hearts perfused with noradrenaline, no significant increase in the activity of nucleolar and nucleoplasmic RNA polymerases is detectable within the time interval (5 min) when the rapid rise of specific radioactivity of RNA occurs. The latter phenomenon thus cannot be considered as an expression of the synthesis of RNA *de novo*.

Many studies have been made on the involvement of polyamines, especially spermidine and spermine, in the metabolism of ribonucleic acids, suggesting that these polycations exert an action either in the processes of RNA synthesis (Kostyo, 1966; Moruzzi *et al.*, 1968; Raina & Jänne, 1970; Snyder *et al.*, 1970; Moruzzi *et al.*, 1975) or in the cytoplasmic phases of protein synthesis (Kay & Pegg, 1973). In the heart a parallel increase in synthesis of RNA and polyamines was shown when the organ was subjected to hypertrophy by work load (Caldarera *et al.*, 1971; Russell *et al.*, 1971; Feldman & Russell, 1972; Caldarera *et al.*, 1974; Moruzzi *et al.*, 1974). Addition of polyamine to perfused rabbit heart increases membrane permeability (Gibson & Harris, 1974) and stimulates gene transcription (Caldarera *et al.*, 1975). Under the action of noradrenaline the specific radioactivities of polyamines and the enzymes responsible for their synthesis change in an orderly sequence. Ornithine decarboxylase, the first enzyme in the only known pathway of spermidine and spermine synthesis in animal tissues, is first stimulated, followed by S-adenosylmethionine decarboxylase and putrescine incorporation into spermidine and spermine. The increase of specific radioactivity of polyamines cannot be totally accounted for, as with RNA, by the increase of precursor uptake (see the Results section). This is consistent with the hypothesis of a specific requirement of the synthesis of these compounds as a response to noradrenaline treatment. However, the timing of the changes is not compatible with the increase of specific radioactivity of RNA. In fact, the highest rate of putrescine incorporation into both spermidine and spermine occurs 10 min after hormone injection, when [³H]ribose incorporation into RNA has returned to near the control value. Nucleoplasmic RNA polymerase activity, on the other hand, appears to parallel the variations in polyamine synthesis. Newly synthesized polyamines therefore cannot support the increase in specific radioactivity of RNA, providing another indication against a synthesis of RNA *de novo* as an early effect of noradrenaline treatment of the heart.

The asserted importance (Huez *et al.*, 1974) of polyadenylation for the efficient translation of mRNA in the cytoplasm suggested to us that cytoplasmic

polyadenylate polymerase may be involved in the possible activation of mRNA species in this sub-cellular compartment as a rapid response of the mechanism of protein synthesis to the hormone. The huge increase in polyadenylate polymerase activity after 30 s of hormone treatment indeed indicates that this activity is one of the first essential metabolic changes evoked by noradrenaline in the heart.

Cyclic AMP appears to be the mediator of the noradrenaline effect. RNA polymerases are not enhanced by administration of the nucleotide to the heart under our experimental conditions (results not shown). In contrast, polyadenylate polymerase activity is stimulated when 10–100 μ M-cyclic AMP is added to the perfusion medium. Pertinent to this effect may be the finding by Pastan & Perlman (1969) that cyclic AMP stimulated the synthesis of tryptophanase in *Escherichia coli* even after mRNA synthesis had been arrested by actinomycin D. At the most effective dose of the cyclic AMP the enzyme activity does not decrease throughout the time of treatment, as it did with noradrenaline. One interpretation may be that the continued direct administration of cyclic AMP may be able to overcome the effect of phosphodiesterase in the heart. The involvement of this mediator, which is known for its effect on phosphorylation/dephosphorylation reactions, leads us to postulate that a specific protein kinase, activated by the nucleotide, is involved in the increase of poly(A) polymerization activity. This is in good agreement with the lack of effect of cyclic AMP added *in vitro* to the polyadenylate polymerase test system.

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