EFFECT OF NOREPINEPHRINE ON SOLUBLE Mn^{++}-STIMULATED POLY(A) SYNTHETASE ACTIVITY AND RIBONUCLEIC ACIDS OF PERFUSED RABBIT HEART.

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Received June 21,1976

Summary: Mn^{++}-stimulated poly(A) synthetase activity solubilized from perfused rabbit heart, whether treated with reserpine or not, is strongly enhanced by addition of norepinephrine to perfusion medium. Incorporation of labeled ribose into RNA, inhibited by reserpine, is restored well above the control level in microsomal RNA and stays inhibited in nuclear RNA, following addition of the hormone.

Recent evidence (1) demonstrates that an enzyme responsible for polyadenylation of primer RNAs is present in the soluble (postmicrosomal) fraction of several mammalian tissues, as was suggested by previous reports (2,3,4). The enzyme from calf thymus has been purified nearly to homogeneity. During its purification, no other fractions showed polyadenylation activity. Furthermore, all of the crude soluble fractions tested after sedimentation on sucrose gradients contained only a single activity (1). Thus, in the absence of evidence to the contrary, it seems correct to use crude tissue extracts in order to gain insight into the function of this enzyme. Recently, we found that a variety of physiological conditions involving synthesis or utilization of mRNA modify Mn^{++}-dependent poly(A) synthetase (5). In the present communication we report the activity of this enzyme, solubilized from perfused rabbit heart, under the effect of norepinephrine alone or together

with reserpine (6). The effect of norepinephrine, with or without reserpine pretreatment, on the incorporation of [^{3}H]ribose into nuclear and microsomal RNA is also determined. The experimental model was chosen because of the intense effect of norepinephrine on mRNA-mediated metabolic processes connected with the supply of new structural and enzymatic proteins (7).

Methods

Male and female (strain) rabbits 3 months old and weighing about 2 Kg were used. The perfusion of the heart was performed without recirculation in a Langendorff apparatus (8). The composition of the perfusion medium was: 154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 5.9 mM $NaHCO_3$, 5.5 mM glucose. The flow rate of perfusion medium was 10 ml/min. The perfusion of the hearts was carried out in every case for 40 min with the perfusion medium to which either nothing, or reserpine, and/or norepinephrine were added. Reserpine (1 mg/15 min) was injected during the first 15 min of perfusion, at a rate of 1 ml/min. Norepinephrine (250 pmoles/ml) was injected at a rate of 1 ml/min 2.5, 5 or 10 min before ending perfusion. The experiments were terminated by freezing the hearts in a Wollenberger clamp cooled in liquid nitrogen, while the hearts were still being perfused. The frozen tissues were used for the assay for poly(A) synthetase activity.

The enzymic extract was the 105,000 g supernatant of a homogenate made in ice cold 0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$, from which nuclei and mitochondria had been centrifuged down (1). The standard test system (9) contained 100 mM Tris-HCl, pH 8.2 (37 °C); 2 mM dithiothreitol; 1 mg/ml of poly(A) (from Miles); 1 mM [8-^{14}C] ATP (1,200-1,500 cpm/nmole); 1 mM $MnCl_2$ and approximately 0.3 mg of protein with water to a final volume of 0.25 ml. After 30 min at 37 °C, the reactions were terminated by addition of ice cold trichloracetic acid to a final concentration of 5 %. The washed acid-insoluble precipitate was dissolved in 88 % formic acid and its radioactivity was determined by scintillation counting (10).

When the incorporation of labeled ribose into RNA of subcellular fractions was studied in reserpinized and norepinephrine treated hearts, 50 μCi/100 ml of [5-^{3}H]ribose (2.9 Ci/mmol) was added to the physiological solution 25 min before ending perfusion. Preparation of subcellular fractions and determination of RNA were as previously reported (11).

Results and discussion

The results of a typical experiment on the effect of norepi-

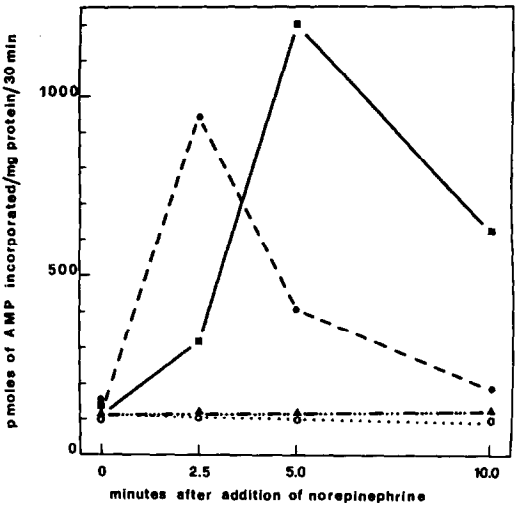

Figure 1. Effect of norepinephrine on soluble Mn^{++}-stimulated poly(A) synthetase activity in normal and reserpinized perfused rabbit heart.
The experimental conditions are described in the text. For each point six animals were used; the S.E.M. was less than 10 %. (▲) normal; (●) + norepinephrine; (○) + reserpine; (■) + reserpine + norepinephrine.

nephrine on soluble Mn^{++}-stimulated poly(A) synthetase activity in perfused rabbit heart are shown in Figure 1. Both in the reserpine treated hearts and in the non-treated ones a manyfold increase of poly(A) synthetase activity occurs as early as 2.5 min after addition of norepinephrine to the perfusion medium. In the hearts not treated with reserpine, the peak effect of norepinephrine on the enzyme occurs at this time. 2.5 min later (5 min after the start of the norepinephrine perfusion) a steep drop of activity begins and after 10 min of treatment the incorporation of AMP into acid insoluble materials falls back in the vicinity of the control values. Reserpine alone had no effect on poly(A) synthetase activity (Figure 1). The hearts treated with reserpine give the same response to the addition of norepinephrine as the non-treated ones, except for the peak of

activity shifting from 2.5 min to 5 min, and reaching higher values than those attained in non-reserpinized hearts.

Figure 2 shows the incorporation of radioactive ribose into nuclear and microsomal RNA of reserpinized rabbit hearts, under the same set of experimental conditions used for assaying polyadenylation activity. Reserpine treatment strongly diminishes the incorporation of [^{3}H]ribose into both nuclear and microsomal RNAs. Administration of norepinephrine to reserpinized hearts causes a steep enhancement of ribose incorporation, showing a peak at 5 min of hormone treatment as was the case for poly(A) synthetase activity. In contrast, the incorporation of labeled ribose into the nuclear fraction of RNA from reserpinized hearts remains diminished after injection of norepinephrine.

The very rapid increase of enzyme activity occuring under the effect of norepinephrine could thus represent one of the first metabolic responses of the target tissue related to a probable increase of protein synthesis, which has been observed under different experimental conditions (7). The shift of the peak shown by the reserpine-treated hearts with respect to the non-treated hearts might be explained by postulating the need for an optimal level of norepinephrine which in the reserpinized hearts, where the endogenous norepinephrine has been depleted by the drug, is reached later than in hearts not treated with reserpine. The quantitative difference between the peak effects of norepinephrine may simply indicate that 2.5 min is not the time at which poly(A) synthetase activity reaches its maximum in non-reserpinized hearts, but that it may lay somewhere between 0 and 5 min.

No definitive interpretation can be given for the dramatic changes of poly(A) synthetase induced by norepinephrine in rabbit perfused heart, since the physiological function of this enzyme in the cytoplasm is so far unknown. However, a tentative explanation consistent with our results can be proposed. It has been shown (12) that polyadenylate chains covalently bound to

mRNA are required for optimal translational efficiency of the genetic message. Under the effect of norepinephrine a rapid increase of cytoplasmic polyadenylation might be a means to improve or restore efficiency of (stored?, aged?) mRNAs the poly(A) chains of which seem to be shortened by ageing (13). Such an effect of norepinephrine would serve to bring about a rapid increase of protein synthesis on demand (7).

Enhanced incorporation of labeled precursor into mRNA and tRNA of mouse liver following *in vivo* injection of norepinephrine has been reported (14). The peak of incorporation of radioactive ribose into microsomal RNA (Figure 2), coinciding with the peak of poly(A) synthetase activity, as compared with the flat line obtained with nuclear RNA in reserpinized heart agrees with but does not by any means prove the idea that a cytoplasmic and not a nuclear handling of RNA occurs as a

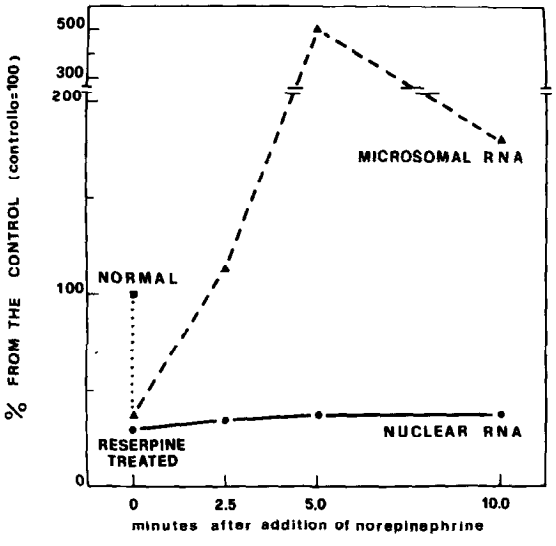

Figure 2. Effect of norepinephrine on specific radioactivity (dpm/mg RNA) of nuclear and microsomal RNA from reserpinized perfused rabbit heart.
The experimental conditions are described in the text. The results are expressed as percentage from the control. The specific radioactivity of the control is: 2,950 ± 105 for nuclear RNA; 3,120 ± 175 for microsomal RNA. For each point six animals were used; the S.E.M. was less than 10 %.

consequence of norepinephrine action. Experiments are underway to determine whether the increased radioactivity of microsomal RNA has in fact to be ascribed to elongation or new formation of poly(A) chains attached to mRNA in the cytoplasm.

References

1. Tsiapalis, C.M., Dorson, J.W. and Bollum, F.J. (1975) J. Biol. Chem. 250, 4486-4496.
2. Klemperer, H.G. (1963) Biochim. Biophys. Acta 72, 416-420.
3. Tsiapalis, C.M., Dorson, J.W., De Sante, D.M. and Bollum, F.J. (1973) Biochem. Biophys. Res. Comm. 50, 737-743.
4. Corti, A., Wilson, J. and Williams-Ashman, H.G. (1974) 9th FEBS Meeting, Budapest.
5. Corti, A. and Drusiani, F. (1976) sent for publication.
6. Alpers, H.S. and Shore, P.A. (1969) Biochem. Pharmacol. 18, 1363-1372.
7. Grand, R.J. (1969) Biochim. Biophys. Acta 195, 252-254.
8. Langendorff, O. (1895) Pfl. Arch. Gesm. Physiol. 61, 291-333.
9. Corti, A., Williams-Ashman, H.G. and Wilson, J. (1975) Boll. Soc. It. Biol. Sper. 51, 256-262.
10. Calvin, H.I., Kosto, B. and Williams-Ashman, H.G. (1967) Arch. Biochem. Biophys. 118, 670-681.
11. Caldarera, C.M., Casti, A., Guarnieri, C. and Moruzzi, G. (1975) Biochem. J. 152, 91-98.
12. Huez, G., Marbaix, G., Hubert, E., Leclercq, M., Nudel, U., Soreq, H., Salomon, R., Lebleu, B., Revel, M. and Littauer, U.Z. (1974) Proc. Nat. Acad. Sci. USA 71, 3143-3146.
13. Brawerman, G. (1973) Mol. Biol. Reports 1, 7-13.
14. Decalonne, J.R., Briquet, M.V., Lambert, R.R. and Wiaux, A.L. (1967) Experientia 23, 365-366.