Vol. 82, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1978 Pages 641-647

ISOPROTERENOL INJECTION ALTERS PROTEIN KINASE DEAE-CELLULOSE PROFILES IN SELECTED RAT TISSUES

Robert Roskoski, Jr., Peter Ngan, Richard Mettenburg and Donald D. Lund

Department of Biochemistry and The Cardiovascular Center
The University of Iowa
Iowa City, IA 52242

Received April 12,1978

SUMMARY

Isoproterenol injection produces cardiac hypertrophy and salivary gland enlargement in rats. After subcutaneous injection (5 mg/kg) twice daily for 10 days, the soluble extracts from these and other tissues were subjected to DEAE-cellulose chromatography and the activity of isozymes I and II of cyclic AMP-dependent protein kinase was measured. The activity of isozyme I is decreased 80% in salivary gland and 40% in liver. No significant changes were observed in kidney, brain or skeletal muscle. In contrast to a previous report, no change was observed in the amount of isozyme I in hypertrophied heart. Furthermore, no changes were observed in the activity of isozyme II in response to isoproterenol injection in any of these tissues.

Cyclic 3',5'-adenosine monophosphate (cAMP)-dependent protein kinase catalyzes the phosphorylation of polypeptidic serine and threonine residues. This class of enzymes is implicated in the regulation of a large number of physiological processes (1). The mechanism of activation by cAMP is shown by the equation $R_2C_2 + 2\ cAMP \rightleftharpoons (R{\cdot}cAMP)_2 + 2C$ where R_2C_2 represents the inactive holoenzyme and R and C represent the regulatory subunit and active catalytic subunit, respectively (2-7). We previously proposed that the R subunit inhibits the C subunit by shielding - either physically or functionally - the active site (8).

Corbin et al. (9) reported that there are two protein kinase isozymes which can be distinguished on the basis of their DEAE-cellulose elution profiles among other criteria. Isozyme I elutes at a lower ionic strength than isozyme II. The isozymes share a common C subunit, but differ in the R subunit (7). The physiological significance of the two enzyme forms is presently unknown. Costa et al. (10) have reported cell cycle specific changes in the activity

0006-291X/78/0822-0641$01.00/0

of the two isozymes in Chinese hamster ovary cells. Lee et al. (11) have also reported developmental changes in the activity of the two enzyme forms during rat testis development.

Byus et al. (12) examined the rat heart protein kinase DEAE-cellulose elution profiles in isoproterenol-induced cardiac hypertrophy. They found an increase in the activity of isozyme I and no change in that of isozyme II. After isoproterenol administration, there is an increase in heart cell size, but no increase in cell number (13). These investigators propose that the relative increase in isozyme I may play an important role in the trophic response. Isoproterenol injection also stimulates DNA synthesis in salivary gland (14) and kidney (15). We measured the cAMP-dependent protein kinase profiles in several rat tissues. We find that the elevation of isozyme I in heart is not a consistent finding. We report, furthermore, that there is a significant decrease in isozyme I activity in liver and in the grossly enlarged salivary gland.

MATERIALS AND METHODS

Isoproterenol Injection. The protocol of Byus et al. (12) for injection, tissue homogenization and chromatography was followed. Six male Sprague Dawley rats (160-210 g) received 5 mg/kg isoproterenol subcutaneously twice daily for 10 days. Six control animals received an equivalent amount of 0.9% NaCl vehicle. The rats were sacrificed by cervical dislocation 12 h after the last injection and the specified tissues were removed, weighed, and stored at -20° C. Pilot experiments showed that protein kinase activity is stable for at least 2 months under these conditions.

DEAE-Cellulose Chromatography. The tissues were disrupted in 3 vol of cold (0-4° for all steps unless otherwise specified) 5 mM Tris-HCl and 1 mM EDTA (pH 7.5) with four 10-sec bursts with a Tekman Tissumizer (Cincinnati, Ohio). After centrifugation at 27,000 xg for 10 min, the supernatant protein was measured by the procedure of Bradford (16) using bovine serum albumin as standard. Then 3.0 mg of protein was applied to a DE-52 cellulose column (0.5 x 4 cm) fabricated from a siliconized Pasteur pipet and 0.3 ml fractions were collected. Protein was eluted with 2 ml of homogenizing buffer followed by an 8 ml (total) linear gradient (0-0.4 M KCl) in buffer. Experimental and control samples were processed tandomly and the order was randomized. Fractions were assayed for protein kinase activity with histone as substrate by the phosphocellulose procedure of Witt and Roskoski (17) except that distilled water was used to elute ATP and metabolites. Since enzyme activity varies with the histone II-A (Sigma Chemical Co.) used, the same lot was used in a given experiment. The [γ-^{32}P]ATP was prepared by the method of Schendell and Wells (18) from carrier free labeled phosphate which was obtained from New England Nuclear.

The type I and II isozymes eluted at approximately 0.10 M and 0.20 M salt, respectively. Activity was 80-95% dependent on exogenous cAMP. The

total number of units (1 nmol ^{32}P/min) derived from the 3.0 mg protein was calculated by addition.

D,L-isoproterenol hydrochloride was obtained from Sigma Chemical Co. The sources of other chemicals has been previously documented (8, 17).

Student's t test was used to determine statistical significance (19).

RESULTS

Wet Weight Responses to Isoproterenol Injection. Isoproterenol injection produced a significant 62% increase in heart weight to body weight ratio (Table 1). Isoproterenol also produced a significant 279% increase in salivary gland weight (Table 1). There was no change, however, in the kidney or liver weights. The increase in cardiac and salivary gland wet weight indicate that the injection of 5 mg/kg isoproterenol (twice daily for 10 days) was effective and the magnitude of cardiac hypertrophy, moreover, was comparable to that reported by Byus et al. (12).

Protein Kinase Profiles in Heart. In agreement with Corbin et al. (9) and Byus et al. (12), isozyme I is the chief form of protein kinase in rat heart. Although isoproterenol injection produced cardiac hypertrophy, we were unable to demonstrate an increase in the activity of isozyme I. To test the notion that strain differences might account for the result, the experiment was also performed with Wistar rats as reported by Byus and coworkers (12). Although a pilot study was suggestive, we failed to find an increase in the isozyme content in Wistar rats even though there was a comparable degree of cardiac hypertrophy. Using a surgical intervention to produce cardiac hypertrophy, under ether anesthesia, a constricting silk ligature was tied around the aorta in one group of six rats. A comparable group received sham surgery consisting of an identical dissection, application, and then removal of the ligature. After 10 days the animals were sacrificed and the heart weight to body weight ratio was significantly ($p < 0.05$) increased 40%. There was no difference, however, in the protein kinase activity profile in response to this intervention (not shown).

Protein Kinase Profiles in Salivary Gland and Liver. In addition to the large increase in weight, isoproterenol injection alters the salivary

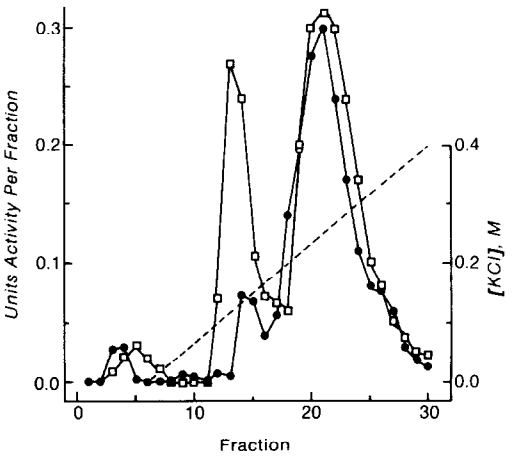

Figure 1. Salivary gland protein kinase profile. Supernatant protein (3.0 mg) was applied to DEAE-cellulose columns, eluted and assayed as described in MATERIALS AND METHODS. The units (nmol/min) per 0.3 ml fraction are plotted. (□- □), control; (0 -- 0) isoproterenol; (---), salt concentration. The total activity associated with isozymes I and II was computed by addition of the activity of the corresponding fractions. The salivary gland isozyme activity was determined from five additional independent measurements to obtain the results given in Table 2.

TABLE 1

ORGAN WEIGHT TO BODY WEIGHT RATIOS

	Control mg/Kg	Isoprel Injection
Heart	3.11 ± 0.14	5.04 ± 0.14[a]
Salivary Gland	1.74 ± 0.046	6.57 ± 0.25[a]
Liver	35.7 ± 0.56	36.5 ± 1.29
Kidney	8.11 ± 0.076	9.12 ± 0.85

The data are the mean ± S.E.M. for 6 animals in each group. The experiment was performed as described in MATERIALS AND METHODS.

[a] $p < .001$ compared with the control

gland protein kinase profile. It decreases the activity of isozyme I by about 80% (Fig. 1; Table 2). Isozyme II activity, however, remains unchanged.

TABLE 2

PROTEIN KINASE ISOZYME ACTIVITY IN RESPONSE TO ISOPROTERENOL INJECTION

Tissue	Isozyme I		Isozyme II	
	Control	Experimental	Control	Experimental
Heart	2.72 ± 0.08	2.50 ± 0.18	0.97 ± 0.11	1.07 ± 0.10
Salivary Gland	0.690 ± 0.053	0.089 ± 0.017[a]	2.14 ± 0.27	1.83 ± 0.22
Liver	0.348 ± 0.04	0.209 ± 0.02[b]	0.459 ± 0.152	0.358 ± 0.017
Skeletal Muscle	1.16 ± 0.04	0.938 ± 0.082	0.572 ± 0.050	0.537 ± 0.039
Kidney	0.267 ± 0.020	0.289 ± 0.032	0.884 ± 0.078	0.916 ± 0.011
Brain	0.213 ± 0.023	0.278 ± 0.050	1.47 ± 0.22	1.49 ± 0.29

The results are expressed as nmols/min per 3.0 mg protein applied to the DEAE-cellulose column.

The mean ± S.E.M. were obtained from 6 animals. Isoproterenol injection and protein kinase activity were performed as described in MATERIALS AND METHODS.

[a] $p < 0.001$

[b] $p < 0.05$

Although the wet weight of the liver is unchanged, isoproterenol injection also produces a 40% decline in the activity of isozyme I. The activity of isozyme II, however, is also unchanged.

Protein Kinase Profiles in Other Tissues. The activity of isozyme I and II remained unchanged in brain (Table 2). Since isoproterenol fails to cross the blood brain barrier, we anticipated that this activity would be unaltered. Isoproterenol injection also failed to significantly alter activity in kidney and skeletal muscle.

DISCUSSION

We draw two conclusions from the present experiments. First, the development of cardiac hypertrophy in response to isoproterenol injection is not invariably associated with an elevation of soluble heart isozyme I activity as determined by DEAE-cellulose chromatography elution profiles. The failure to

find an increase is not the result of dissociation of holoenzyme since there is little activity eluting as free catalytic subunit (prior to isozyme I). In addition to soluble protein kinase activity, possible activity associated with the membrane fraction was solubilized with Triton X-100 using the methodology of Corbin et al. (20). Less than 10% of total activity was associated with this fraction. In agreement with these investigators, moreover, most of it was eluted from DEAE-cellulose columns in fractions corresponding to isozyme II. Although we obtained similar results with Sprague Dawley and Wistar rats, other unidentified physiologic parameters may be responsible for the difference between our results and those of Byus et al. (12). Since isoproterenol injection, for example, produces localized heart cell neurosis and tissue death within 24 h (21), some heart regions or some animals may not exhibit an elevation of soluble isozyme I activity. It is possible that isozyme I is appreciably activated in vivo, but the activity ratios (± cAMP) would have to be measured under conditions corresponding to those in situ (22) to demonstrate this possibility. That the protein kinase profiles are the result of complex regulatory phenomena is suggested by species variability. Rat heart, for example, contains mainly isozyme I; steer, isozyme II; and rabbit about 50% of each (22).

The second finding is that isozyme I declines in salivary gland and liver after isoproterenol injection. The former undergoes hypertrophy and hyperplasia. There is no change in the weight of the latter tissue. Even though there is an increase in DNA synthesis in kidney (15), no alterations in enzyme profiles were observed. Similarly, no changes were seen in brain. These findings were consistently found in Wistar as well as Sprague-Dawley rats.

Additional experimentation will be required to learn the mechanism whereby isoproterenol injection and the subsequent β-adrenergic receptor stimulation mediate a decrease in the relative specific activity of protein kinase isozyme I in some tissues and its failure to alter that of isozyme II.

ACKNOWLEDGMENT

This work was supported by a Special Postdoctoral Fellowship to D. D. L. (HL-05529) and a Program Project Grant (HL-014388) from the U. S. Public Health Service. We thank Dr. Jonathan J. Witt for valuable discussion.

REFERENCES

1. Greengard, P. (1978) Science, 146-152.
2. Tao, M., Salas, M. L. and Lipmann, F. (1970) Proc. Nat. Acad. Sci. USA 67, 408-414.
3. Gill, G. N. and Garren, L. D. (1970) Biochem. Biophys. Res. Commun. 39, 335-343.
4. Kumon, A., Yamamura, H. and Nishizuka, V. (1970) Biochem. Biophys. Res. Commun. 41, 1290-1297.
5. Reimann, E. M., Brostrom, C. O., Corbin, J. D., King, C. A. and Krebs, E. G (1971) Biochem. Biophys. Res. Commun. 42, 187-194.
6. Ehrlichman, J., Rubin, C. S. and Rosen, O. M. (1973) J. Biol. Chem. 248, 7607-7609.
7. Hofmann, F., Beavo, J. A., Bechtel, P. J. and Krebs, E. G. (1975) J. Biol. Chem. 250, 7795-7801.
8. Witt, J. J. and Roskoski, R., Jr. (1975) Biochemistry 14, 4503-4507.
9. Corbin, J. D., Keely, S. L. and Park, C. R. (1975) J. Biol. Chem. 250, 218-225.
10. Costa, M., Gerner, E. W. and Russell, D. H. (1976) J. Biol. Chem. 251, 3313-3319.
11. Lee, P. C., Radloff, D., Schweppe, J. S. and Jungmann, R. A. (1976) J. Biol. Chem. 251, 914-921.
12. Byus, C. V., Chubb, J. M., Huxtable, R. J. and Russell, D. H. (1976) Biochem. Biophys. Res. Commun. 73, 694-702.
13. Stanton, H. C., Brenner, G. and Mayfield, E. D. (1969) Amer. Heart J. 77, 72-80.
14. Barka, T. (1965) Exp. Cell Res. 37, 662-679.
15. Malamud, D. and Malt, R. A. (1971) Lab. Invest. 24, 140-143.
16. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254.
17. Witt, J. J. and Roskoski, R., Jr. (1975) Anal. Biochem. 66, 253-258.
18. Schendell, P. F. and Wells, R. D. (1973) J. Biol. Chem. 248, 8319-8321.
19. Snedecor, G. W. and Cochram, W. G. (1967) Statistical Methods pp. 69-71, 549. The Iowa State University Press, Ames, Iowa.
20. Corbin, J. D., Sugden, P. H., Lincoln, T. M. and Keely, S. C. (1977) 252, 3854-3861.
21. Fleckenstein, A., Janke, J., Doring, H. J. and Leder, O. (1975) In A. Fleckenstein and G. Rona (eds.) Recent Advances on Cardiac Structure and Metabolism. Vol. 6; University Park Press, Baltimore, pp. 21-32.
22. Corbin, J. D. and Keely, S. L. (1977) J. Biol. Chem. 252, 910-918.