INCREASE IN TYPE I ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE DURING ISOPROTERENOL-INDUCED CARDIAC HYPERTROPHY

Craig V. Byus, James M. Chubb, Ryan J. Huxtable
and Diane H. Russell

University of Arizona College of Medicine
Department of Pharmacology
Tucson, Arizona 85724

Received September 13,1976

SUMMARY

The amount of type I and type II cyclic AMP-dependent protein kinase present in the rat heart was determined at various times during isoproterenol-induced cardiac hypertrophy. Wistar rats were injected twice daily with isoproterenol (5 mg/kg, s.c.) for 2, 5 or 10 days. Cardiac weight increased gradually over the 10-day period of drug administration, and by day 10, heart weight was 156% of control. Following the cessation of isoproterenol administration, the cardiac weight regressed toward the control value by day 15. An increase in the specific activity of type I protein kinase to 197% of control occurred by day 10. The specific activity of type II protein kinase did not change significantly during either the hypertrophy or regression stage. The increase in the specific activity of type I protein kinase during a chemically-induced trophic response of the heart may indicate that type I cyclic AMP-dependent protein kinase plays a regulatory function in this process.

The characterization of soluble cyclic AMP-dependent protein kinase(s) (EC 2.7.1.70) from a number of mammalian tissues indicates they are composed generally of two regulatory subunits and two catalytic subunits (R_2C_2) (1-5). Cyclic AMP binds to the regulatory subunit, dissociating the holoenzyme and freeing the active catalytic subunit. Rat cardiac muscle contains at least two types of cyclic AMP-dependent protein kinase(s) (6) that can be differentiated by DEAE-cellulose chromatography. Type I elutes at a lower salt concentration than the type II kinase. Both kinases are thought to contain the same catalytic but different regulatory subunits (7), are found in the cytosol, and exhibit similar properties in terms of substrate activity, nucleotide specificity, and K_m for ATP (8). After activation, there is some evidence of nuclear translocation of the free catalytic subunit (9).

Distinct physiological functions for the type I and type II kinases have not been demonstrated; however, the different dissociation-reassociation properties of the two enzymes (6) and their unique regulatory units suggest separate regulatory functions in the cell. Costa et al. (10) have recently reported cell cycle-specific changes in the activity of type I and type II protein kinases in Chinese hamster ovary cells. Type I activity was high during mitosis, whereas an increase in the total amount of type II gained in middle to late G_1 phase of the cell cycle. Puromycin blocked both the entrance of the cells into S phase and the synthesis of type II kinase (10). Lee et al. (11) have also reported changes in the relative amounts of type I and type II kinase in the rat testis during postnatal development.

In light of this work, we have measured the amounts of type I and type II protein kinase during the hypertrophy and regression of cardiac tissue induced by isoproterenol treatment. In this animal model, the increase in organ mass is mainly a result of cell enlargement rather than cell division (12). There are major increases in RNA and protein content with negligible increases in DNA (12). We suggest that the large increase in the amount of type I protein kinase which occurs during cardiac hypertrophy may play an important role in this trophic response.

MATERIALS AND METHODS

Calf thymus histones (mixture) was obtained from Calbiochem, Inc. D,L-isoproterenol hydrochloride, cyclic AMP, ATP and DEAE-cellulose were purchased from Sigma, Inc. [γ-^{32}P]-ATP (7 Ci/mM) was obtained from New England Nuclear. 1-Methyl, 3-isobutylxanthine (MIX) was obtained from Aldrich Chemical Co., Inc.

Induction of cardiac hypertrophy. Male Wistar rats (160-210g) were used in all experiments. The animals were injected subcutaneously twice daily for 2, 5, or 10 days with 5 mg/kg isoproterenol. Controls were injected in a similar manner with an equal volume of 0.9% NaCl. The rats were sacrificed in the morning, 12 hr after the previous injection of isoproterenol on days 2, 5, and 10 following initiation of drug treatment. Rats were also sacrificed on day 15, having received isoproterenol for the first 10 days and no drug for the following 5 days. The injection schedule was arranged so that all animals were sacrificed over a 4-day period. This procedure resulted in all animals being approximately the same age and weight when sacrificed.

DEAE-cellulose chromatography. The animals were decapitated and the hearts homogenized in 3 vol of cold 5 mM Tris, pH 7.5, containing 1 mM EDTA. The homogenate was centrifuged at 27,000 x g for 10 min and 1 ml of the supernatant

Table 1

HEART WEIGHT DURING CARDIAC HYPERTROPHY AND REGRESSION

Day	Number of Animals in Each Group	Wet Weight (mg)	Dry Weight (mg)
Control	4	645 ± 40	184 ± 10
2	3	859 ± 20*	205 ± 10
5	3	975 ± 30*	257 ± 6*
10	3	1005 ± 29*	270 ± 8*
15	4	836 ± 57*	236 ± 13*

*$p < 0.05$.

Animals were administered isoproterenol (5 mg/kg, s.c.) twice daily for 10 days (or until sacrificed). Controls were administered an equal volume of 0.9% NaCl. Animals were sacrificed at days 2, 5 and 10 during the development of cardiac hypertrophy, and at day 15, 5 days following the last injection of isoproterenol. Heart were weighed, homogenized in 3 vol of 5 mM Tris, pH 7.5, containing 1 mM EDTA and centrifuged. The pellet was dried for 1 week in a vacuum desicator and the dry weight was determined. Data shown represent the mean ± S.E.M.

---

was applied to DEAE-cellulose columns (0.7 x 12 cm), equilibrated in 5 mM Tris, pH 7.5, containing 1 mM EDTA. The columns were washed with 30 ml of buffer prior to eluting the protein kinases with 80 ml of a linear NaCl gradient (0-0.4 M). Fractions (1.5 ml) were collected throughout at 4 °C.

Type I and type II protein kinase assays. The amounts of type I and type II cyclic AMP-dependent protein kinase were determined after fractionation by DEAE-cellulose chromatography. The assay for protein kinase activity was performed essentially as described by Corbin et al. (13) within 1 hr following completion of chromatography. Fifty µl from each fraction were assayed in a total volume of 100 µl containing 0.05 M sodium-phosphate, pH 6.8, 0.5 mM MIX, 100 µg mixed calf thymus histone, 0.15 mM [γ-^{32}P]-ATP (0.5-1 µCi) and 5 µM cyclic AMP. The reaction was run for 10-15 min at 30 °C and was terminated by spotting 60 µl onto Whatman paper filters (3 mm) which were dropped into 15% cold trichloroacetic acid (TCA). The filters were washed for 15 min

followed by an additional two washes in 5% TCA. They were then immersed briefly in 95% ethanol, dried, and the radioactivity determined by liquid scintillation. Protein kinase activity was expressed as units/μl (1 unit of kinase activity is defined as that amount of enzyme which catalyzes the transfer of 1 μmole of phosphate from ATP to histone in 1 min).

Statistical analyses were performed on a Wang Series 700 Programmable Calculator. Values as indicated in the test are expressed as means ± S.E.M., and a difference with probability of < 0.05, as calculated by the student t test, was considered significant.

RESULTS

Isoproterenol-induced cardiac hypertrophy

Twice-daily injections of isoproterenol, 5 mg/kg, s.c., caused an increase in heart weight already apparent by day 2 as shown in Table 1. This initial increase in heart weight was due mainly to fluid retention since the dry weight was not significantly different from control on day 2. By day 5, wet and dry weights were significantly greater than controls and continued to increase until day 10 when drug administration was discontinued. On day 10, the cardiac wet weight of treated animals was 156% of control. Continued administration of isoproterenol beyond 10 days resulted in slightly greater cardiac enlargement, but was associated with severe mortality (12,14). In this study, following the cessation of isoproterenol administration on day 10, the heart weight decreased toward the control value (Table 1).

Separation of protein kinases

The major forms of the heart soluble protein kinase were separated by DEAE-cellulose chromatography (Fig. 1, control). The initial flow-through fractions contained free catalytic subunit, the activity of which was not stimulated by cyclic AMP. The cyclic AMP-dependent protein kinases, type I and type II were eluted from the columns at NaCl concentrations of 0.06 - 0.08 M and 0.20-0.24 M, respectively. A small amount of protein kinase which was inhibited slightly by cyclic AMP was eluted at a NaCl concentration of 0.002-0.01 M. Other investigators have not reported the presence of this small peak of activity (6), perhaps because they used chromatography conditions insufficient to separate it from the type I kinase. It is also possible

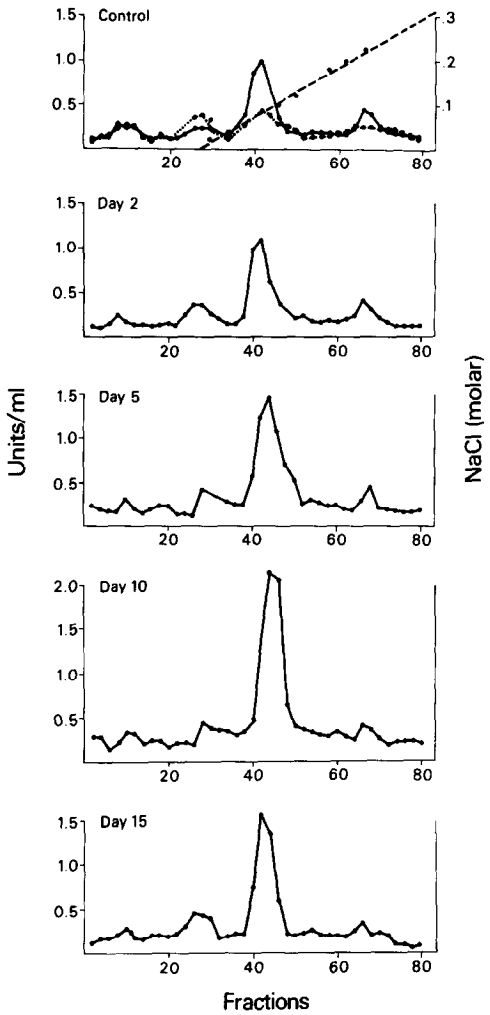

Figure 1. DEAE-cellulose chromatography of protein kinase during cardiac hypertrophy and regression of hypertrophy. Wistar rats were administered isoproterenol (5 mg/kg, s.c.) twice daily for 10 days (or until sacrificed). Controls were administered an equal volume of 0.9% NaCl. On days 2, 5, 10 and 15 (5 days after the last injection of isoproterenol), hearts from treated and control rats were homogenized in 3 vol 5 mM Tris, pH 7.5, containing 1 mM EDTA and centrifuged at 27,000 x g for 10 min. One ml of the supernatant (12 mg protein/ml) was applied to DEAE-cellulose columns (0.7 x 12 cm) which had been equilibrated in the same buffer. After washing the column with 30 ml of buffer, a linear gradient (0 to 0.4 M NaCl) was started. Each gradient flask contained 40 ml. The fractions (1.5 ml) were assayed for protein kinase in the presence (●——●) and absence (●-----●) of 5 μM cAMP. Type I and type II cAMP-dependent protein kinase eluted at NaCl concentrations of 0.06-0.08 M and 0.20-0.24 M, respectively. A third cAMP-independent fraction eluted at a NaCl concentrationof 0.002-0.01 M. The recovery of the enzyme activity was 70-80% for every column. The data shown are representative of determinations from 2 control animals and 4 isoproterenol-treated animals on days 2, 5, 10, and 15.

that this kinase loosely bound to DEAE is produced from one of the other forms of protein kinase by proteolytic digestion or by dephosphorylation.

Protein kinase activity during the development and regression of cardiac hypertrophy

The amount of the protein kinases was determined during the development of isoproterenol-induced cardiac hypertrophy on days 2, 5, and 10, and also during the regression of hypertrophy on day 15, 5 days following the last injection of isoproterenol. The amount of free catalytic subunit and the small protein kinase peak inhibited by cyclic AMP remained unchanged relative to the control levels throughout the hypertrophy and regression stages (Fig. 1) Protein kinase type I activity was not signficantly different from control on day 2, but by day 5, the amount was 161% of control and by day 10, 197% of control (Fig. 1). Five days following cessation of the drug, protein kinase type I was still 140% of control. The amount of type II protein kinase did not change significantly during this process (Fig. 1).

Protein kinase type I activity and heart weight

Figure 2 shows the relationship between the heart weight/body weight ratio and protein kinase type I activity during the development and regression of isoproterenol-induced cardiac hypertrophy. The heart weight/body weight ratio increased over the 10-day period of isoproterenol administration to a maximum ratio of 5.5 at day 10. Upon cessation of the drug treatment, the ratio regressed toward control value. Protein kinase type I activity closely followed the increase and decrease in heart wieght/body weight ratio during the hypertrophy and regression stages following day 2. On day 2, protein kinase type I activity was not significantly different from control, yet the ratio was elevated. The lack of association between the heart weight/body weight ratio and protein kinase type I activity prior to day 2 can be explained by the finding that this early increase in heart weight was attributable to edema, since dry weight had not yet increased significantly (Table 1).

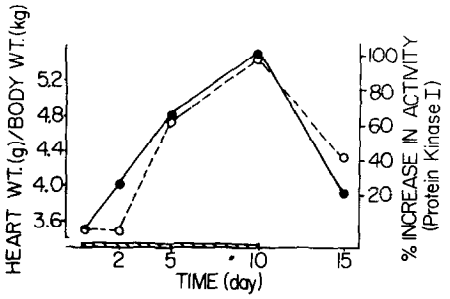

Figure 2. Cyclic AMP-dependent protein kinase type I activity and heart weight/body weight ratio during the development and regression of cardiac hypertrophy. Isoproterenol (5 mg/kg, s.c.) was administered twice daily for 10 days (or until sacrificed). On day 2, 5, and 10 during the development of cardiac hypertrophy and on day 15, 5 days following the last injection of isoproterenol, wet heart weight and body weight were determined in treated and control animals. Protein kinase activity was also determined as described in Methods. ▨, duration of isoproterenol administration; ●——●, type I protein kinase activity; ●-----●, heart weight/body weight. Data shown for heart weight/body weight ratio represent the mean (± S.E.M. fall within circles) for 3-4 animals for each time point. Ratio is significantly greater ($p < 0.05$) than control on day 2, 5, 10 and 15. Data for protein kinase are representative of 2 control and 4 isoproterenol-treated animals for each time point.

DISCUSSION

Horwood and Singhal (15) recently reported isoproterenol-induced changes in total soluble myocardial kinase activity. They found that supernatant kinase activity, in agreement with our data (Fig. 2), increased coincidentally with the elevated heart growth rate following isoproterenol treatment. The time-course and degree of cardiac hypertrophy reported by us, however, is substantially different from that observed by Horwood and Singhal (Table 1, Fig. 2). They found the maximal increase in heart weight occurred between 18 and 24 hr and 2 to 4 days of isoproterenol treatment, while we report continual increases in both wet and dry weight of the heart during the 10-day period (Table 1). These differences may be attributable to the use of a different strain of rat as well as to the use of larger animals (+ 25g). In addition, we employed a different injection schedule administering isoproterenol every 12 hr instead of every 24 hr.

Cyclic AMP-dependent protein kinase(s) have been implicated in regulating several heart functions including the mediation of inotrophic and chronotrophic responses and glycogenolysis (16,17). These kinases may also be involved in the regulation of RNA and protein synthesis by mediating specific gene expression and enzyme induction (18,19). The presence of at least two cyclic AMP-dependent protein kinases with dissimilar biochemical properties suggests that each may have specific regulatory roles in the cell. We have observed that only the specific activity of type I protein kinase increases during the marked hypertrophy of the rat heart resulting from isoproterenol treatment (Fig. 1, Table 1).

In our model of isoproterenol-induced hypertrophy, the increase in organ mass is predominantly a result of an increase in myofibril protein synthesis involving increased ribosomal RNA synthesis without substantial synthesis of DNA (12). Type I kinase may be involved with the hypertrophic response or may be necessary for the maintanence of elevated glucose metabolism and other catabolic processes. Recent work by Costa et al. (10) and Lee et al. (11) as well as the specific accumulation of type I kinase in the heart all suggest that the synthesis of type I and type II protein kinases is controlled independently.

ACKNOWLEDGEMENTS

This work was supported by USPHS grants CA-14783 and CA-17094 from the National Cancer Institute [DHR] and by USPHS grant HL-19394 (RJH). Dr. Byus is the recipient of PHS Research Fellowship CA-05063 from the National Cancer Institute. Dr. Russell is the recipient of the National Cancer Institute Research Career Development Award CA-00072.

REFERENCES

1. Corbin, J.D., Brostrom, C.O., Alexander, R.L. and Krebs, E.G. (1972) J. Biol. Chem, 247, 3736-3743.
2. Gill, G.N. and Garren, L.D. (1970) Biochem. Biophys. Res. Commun, 39, 335-343.
3. Tao, M., Salas, M.L. and Lipman, F. (1970) Proc. Nat. Acad. Sci. USA 67, 408-414.

4. Rubin, C.S., Erlichman, J. and Rosen, O.M. (1972) J. Biol. Chem, 247, 36-44.
5. Kumon, A., Yamamura, H. and Nishizuka, V. (1970) Biochem. Biophys. Res. Commun, 41, 1290-1297.
6. Corbin, J.D., Keely, S.L. and Park, C.R. (1975) J. Biol. Chem, 250, 218-225.
7. Bechtel, P.J., Beavo, J.A., Hofman, F., Dills, W.L. and Krebs, E.G. (1975) Fed. Proc, 34, 2251.
8. Keely, S.L., Corbin, J.D. and Park, C.R. (1975) J. Biol. Chem, 250, 4832-4840.
9. Jungmann, R.A., Lee, S.D. and DeAngelo, A.B. (1975) Advances in Cyclic Nucleotide Research, Vol. 5, pp. 281-306, (eds., G.I. Drummond, P. Greengard, and G.A. Robison) Raven Press, New York, NY.
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. Stanton, H.C., Brenner, G. and Mayfield, E.D. (1969) Amer. Heart J., 77, 72-80.
13. Corbin, J.D. and Reimann, E.M. (1974) Methods in Enzymology, Vol. 38 (eds., J.G. Hardman and B. O'Malley) pp. 287-290, Academic Press, New York.
14. Chubb, J., Huxtable, R. and Bressler, R. (1975) Pharmacologist 17, 214.
15. Horwood, D.M. and Singhal, R.L. (1976) J. Molec. and Cell. Cardiol, 8, 29-38.
16. Robison, G.A., Butcher, R.W. and Sutherland, E.W. (1971) Cyclic AMP, pp. 164-172, Academic Press, New York.
17. Brooker, G. (1975) Advances in Cyclic Nucleotide Research, Vol. 5, pp. 435-452, (eds., G.I. Drummond, P. Greengard and G.A. Robison) Raven Press, New York, NY.
18. Wicks, W.D. (1975) Advances in Cyclic Nucleotide Research, Vol. 4, pp. 335-438, (eds., P. Greengard and G.A. Robison) Raven Press, New York, NY.
19. Jungmann, R.A. and Russell, D.H. (1976) Life Sciences, in press.