Biochimica et Biophysica Acta, 972 (1988) 110–112
Elsevier

BBA 10221

BBA Report

Protein kinase C (calcium/phospholipid-dependent protein kinase) in developing chick heart: selective localization to atrium versus ventricle and changes in activity levels during cardiogenesis

Robert W. Wrenn, Mary A. Barrow and Mary E. Redmond

Department of Anatomy, Schools of Medicine and Biomedical Graduate Studies, Medical College of Georgia, Augusta, GA (U.S.A.)

(Received 8 August 1988)

Key words: Protein kinase C; Protein localization; Protein activity; Cardiogenesis; (Chick heart)

Activity levels of calcium/phospholipid-dependent protein kinase were examined in preparations of atria and ventricles from embryonic chick hearts at various stages of development. Activity of protein kinase C was much higher in atria than ventricles. Protein kinase C activity underwent a progressive increase in atria during cardiogenesis, being highest just prior to hatching, followed by a profound decrease in activity after hatching. In constrast, activity of cyclic AMP-dependent protein kinase (protein kinase A), while also higher in atria than ventricles, remained relatively constant at the developmental stages examined, likewise decreasing following hatching. These progressive changes in atrial protein kinase C activity suggest a potential regulatory role for this enzyme in cardiogenesis.

Protein kinase-catalyzed phosphorylation of cellular proteins is considered to be a major regulatory mechanism involved in control of metabolic processes [1]. The potential involvement of protein phosphorylation in the regulation of normal embryonic development has been suggested [2], but is not clearly established. Recently, a calcium/phospholipid-dependent protein kinase system (protein kinase C) has become extensively studied as a potential regultor of normal cellular functions [3,4], as well as of aberrant activity such as mitogenesis [5]. This enzyme system has, however, been less examined during the course of normal development. The present studies were designed to determine activity levels of protein kinase C in preparations from the atria and ventricles of hearts from chick embryos at various developmental stages. Activity levels of cyclic AMP-dependent protein kinase (protein kinase A) were also examined at these stages. Activity of protein kinase C was found to be greatly concentrated in the atrial preparation, with little activity noted in ventricle. A developmental pattern of increasing activity of protein kinase C during the course of normal cardiogenesis was also found in the atrial preparations, in contrast to relatively constant levels of protein kinase A activity over the identical period.

Fertilized Arbor Acre chicken eggs (Seaboard Hatchery, Athens, GA) were incubated in a forced-draft incubator maintained at 99% humidity and 39°C. Hearts from embryos or newly hatched chicks were removed at various developmental stages (as indicated in individual figure legends), trimmed of the great vessels, and sep-

Correspondence: R.W. Wrenn, Department of Anatomy, Schools of Medicine and Biomedical Graduate Studies, Medical College of Georgia, Augusta, GA 30912, U.S.A.

arated into atria and ventricles. Tissue was homogenized in 10 vol. of 20 mM Tris-Cl (pH 7.5) containing 100 μg/ml aprotinin, 2 mM EDTA and 50 mM 2-mercaptoethanol (solution A). The homogenate was centrifuged at 30000 × *g* for 30 min and the supernatant was assayed for protein kinase activities. The method used to assay cAMP-dependent protein kinase (protein kinase A) was basically that of Kuo [6], with some modification in the final processing of the assay. The reaction was carried out at 30°C in 0.1 ml of 50 mM Tris-Cl (pH 7.5) containing mixed histone (40 μg; Sigma), $MgCl_2$ (10 mM) with or without 8-bromo-cAMP (10 μM) and 40–50 μg of sample protein. Activity of the calcium/phospholipid-dependent protein kinase (protein kinase C) was in a similar reaction mixture using lysine-rich histone (type II-S, Sigma) as exogenous substrate, in the presence of phosphatidylserine (5 μg) with and without $CaCl_2$. The reaction was initiated by the addition of [γ-^{32}P]ATP (1 nmol, $(0.5–1.0) \cdot 10^6$ cpm) and allowed to continue for 5 min. The reaction was terminated by the addition of 45 μl of galcial acetic acid and the entire assay mixture was spotted into a 1/4 × 1/4 inch square of Whatman P81 chromatography paper, which was then sequentially washed with 30% acetic acid, 15% acetic acid and acetone. After drying, the squares containing individual samples were placed in scintillation vials and the radioactivity was measured by Cerenkov counting.

Initial studies revealed a dramatic partitioning of protein kinase C activity to the soluble fraction obtained from atria as opposed to the same preparation from ventricle (Table I). These results, obtained in a relatively late (19 day) embryonic stage, were subsequently confirmed in rat heart although absolute levels of protein kinase C activity were somewhat lower than those noted in the chick heart (Table I). Little activity of this enzyme was found in the membrane fraction obtained from either atria or ventricles (data not shown), and further results presented were obtained in soluble preparations. Experiments involving various detergent solubilization treatments of the membrane fractions possibly to reveal latent protein kinase C activity are currently underway.

Further experiments were carried out to assess activity of protein kinase C at various developmental stages. Atrial protein kinase C activity steadily increased during the period from developmental day 4 to day 19, then dropped sharply following hatching (Fig. 1, ○). Ventricular protein kinase C activity was markedly lower than activity of this enzyme noted in atria at all developmental stages studied, and was relatively constant except for a slight (but statistically significant, $P < 0.05$) increase at day 12 (Fig. 1, △). The changes noted in protein kinase C activity at various developmental stages were not attributable to altered basal histone kinase activity, since such basal activity remained relatively constant during the course of cardiogenesis in both atria and ventricle (Fig. 2).

TABLE I

ATRIAL AND VENTRICULAR ACTIVITY LEVELS OF CALCIUM/PHOSPHOLIPID-DEPENDENT PROTEIN KINASE IN PREPARATIONS FROM 19-DAY EMBRYONIC CHICK AND ADULT RAT

Tissues were prepared and analyzed for protein kinase C activity as described. Data given represents the mean ± S.E. of the mean from four separate experiments.

	Protein kinase C (pmol ^{32}P/min per mg)	
	atrium	ventricle
Chick (19-day embryo)	63.3 ± 9.8	4.5 ± 0.8
Rat (adult)	18.9 ± 1.5	2.2 ± 0.7

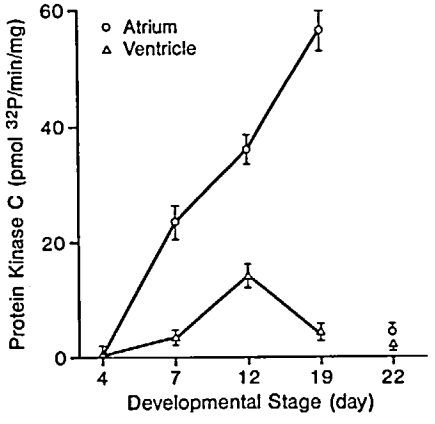

Fig. 1. Atrial (○) and ventricular (△) activity levels of protein kinase C at various stages of embryonic development. Tissue preparation and assay conditions were as described in the text. Each point represents the mean ± S.E. of the mean from three separate experiments.

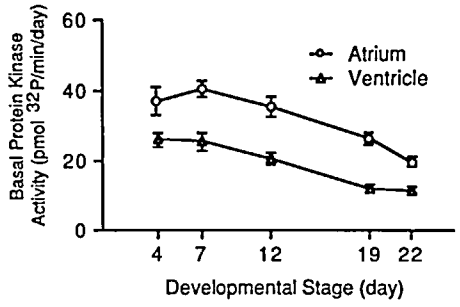

Fig. 2. Basal (unstimulated) protein kinase activity levels in preparations of atria (○) or ventricle (△) at various stages of embryonic development. Tissue preparation was as described in the text. Protein kinase activity was measured in the absence of exogenous activators. Each point represents the mean ± S.E. of the mean from three or four separate experiments.

Activity of cyclic AMP-dependent protein kinase (protein kinase A) was examined in atrial and ventricular preparations from several developmental stages in the chick, as well as in rat. As detailed in Table II, protein kinase A activity in the soluble fraction from chick atria was decidedly greater than activity found in the ventricle of hearts from 7-, 12- and 19-day chick embryos. Activity of protein kinase A in both atria and ventricles remained essentially constant at the developmental stages examined, dropping sharply immediately following hatching. While protein kinase A activity was higher in the atrial preparation than the ventricular preparation from rat heart, the difference was not of the magnitude noted in the chick preparations (Table II).

TABLE II

ATRIAL AND VENTRICULAR ACTIVITY LEVELS OF CYCLIC AMP-DEPENDENT PROTEIN KINASE AT VARIOUS STAGES OF DEVELOPMENT IN CHICK AND ADULT RAT

Tissues were prepared and assayed for protein kinase A activity as described. Data presented represents the mean ± S.E. of the mean from three or four separate experiments.

	Day (before hatching)	Protein kinase A (pmol ^{32}P/min per mg)	
		atrium	ventricle
Chick	7	45.7 ± 6.2	7.0 ± 2.7
	12	47.8 ± 6.7	11.0 ± 2.4
	19	45.0 ± 5.1	8.6 ± 1.3
	0	17.1 ± 5.1	2.1 ± 0.5
Rat		34.0 ± 2.8	21.1 ± 3.5

The developing chick heart provides a very suitable model for study of the potential role of protein phosphorylation systems in ontogenesis. The relative differences in activity levels of protein kinase C and protein kinase A during the course of cardiac development suggests distinct regulatory and functional roles for these enzymes. The results obtained in this study are in line with earlier work on fetal mammalian brain, which noted higher levels of protein kinase C in developing neural tissue, reaching a peak at birth and declining postnatally [8]. Our findings are consistent with an earlier report nothing higher levels of protein kinase A in fetal relative to newborn heart [9]. Further work is underway to characterize the cardiac substrate proteins for protein kinase C and protein kinase A at various developmental stages, as well as to attempt to eludicate possible differential developmental role(s) for these protein phosphorylation systems in atrium versus ventricle.

Supported by USPHS Grant PO 1 HL 36059. The authors gratefully acknowledge the technical assistance of Lynn Mays.

References

1 Krebs, E.G. and Beavo, J.A. (1979) Annu. Rev Biochem. 48, 923–259.
2 Kuo, J.F. (1975) Proc. Natl. Acad. Sci. USA 72, 2256–2259.
3 Wrenn, R.W. and Wooten, M.W. (1984) Life Sci. 35, 267–276.
4 Kojima, I., Kojima, K. and Rasmussen, H. (1985) J. Biol. Chem. 260, 4248–4256.
5 Castagna, M., Takai, Y., Kaibuchi, K., Sana, K., Kikkawa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
6 Kuo, J.F. (1974) Proc. Natl. Acad. Sci. USA 71, 4037–4041.
7 Kuo, J.F., Andersson, R.G.G., Wise, B.C., Mackerlova, L., Salomonsson, I., Brackett, N.L., Katoh, N., Shoji, M. and Wrenn, R.W. (1980) Proc. Natl. Acad. Sci. USA 77, 7039–7043.
8 Wise, B.C., Anderson, R.G.G., Mackerlova, L., Raynor, R.L., Salomonsson, I. and Kuo, J.F. (1981) Biochem. Biophys. Res. Commun. 99, 407–413.
9 Hosey, M.M. (1982) Biochim. Biophys. Acta. 690, 106–116.