Vol. 138, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1986 Pages 387-391

AGE-DEPENDENT CHANGES IN EXPRESSION OF ALPHA$_1$-ADRENERGIC RECEPTORS IN RAT MYOCARDIUM

William Schaffer
and
R. Sanders Williams*

Departments of Medicine and Physiology
Post Office Box 3945
Duke University Medical Center
Durham, North Carolina 27710

Received April 22, 1986

The expression of alpha$_1$-adrenergic receptors within ventricular myocardium of rats ranging in age from 21 days of fetal life to 24 months after birth was measured from [^{125}I] 2-(β hydroxy phenyl) ethylaminomethyl tetralone binding isotherms. No difference was observed in binding affinity between any of the age groups studied. The number of alpha$_1$-adrenergic receptors was found to be 60-120% higher in membranes from fetal or immature rats up to 25 days of age when compared with adult animals. The increased expression of alpha$_1$-adrenergic receptors in the developing heart relative to that observed in adult heart is consistent with the hypothesis that alpha$_1$-adrenergic receptor stimulation may modulate protein synthesis and growth in mammalian myocardium.

Alpha$_1$-adrenergic receptors are present in the mammalian heart (1) and, in addition to the more familiar beta adrenergic receptors, mediate inotropic and chronotropic responses to catecholamine stimulation (2). In addition to acute physiological responses, stimulation of α_1AR has been observed to promote hypertrophy in isolated myocytes (3), suggesting an additional physiologic function of this subclass of receptors.

If, indeed, cardiac α_1AR serve as growth promoting receptors in cardiac myocytes in vivo, we hypothesized that these receptors would be expressed to a greater extent during periods of development characterized by rapid cardiac growth. In order to test this hypothesis we compared the density of α_1AR

*To whom correspondence should be addressed.

Supported by NIH grant HL31101 and by a grant from the PepsiCo Foundation. Dr. Williams is an Established Investigator of the American Heart Association.

Abbreviations used in the text: α_1AR, alpha$_1$-adrenergic receptors; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; $MgCl_2$, magnesium chloride; [^{125}I]-HEAT, [^{125}I] 2-(β hydroxy phenyl) ethylaminomethyl tetralone.

0006-291X/86 $1.50

present in membrane fractions of ventricular myocardium isolated from fetal, neonatal, weanling, mature and senescent rats.

MATERIALS AND METHODS

Long-Evans rats of known gestational age or birth date were obtained from Charles River Laboratories, and 24-25 month old Long-Evans rats were obtained from the Center for the Study of Aging and Human Development at Duke University Medical Center. Adult rats were anesthetized by 100% CO_2 inhalation and killed by a blow to the head. Fetal (21 days gestation) and young rats were killed by decapitation. Hearts were removed and ventricles were dissected free from atria and great vessels, washed in 0.9% NaCl, 10 mM Tris-HCl pH 7.5 and frozen in liquid N_2 and stored at -80°C for later use.

For experiments involving fetal, 1 day, 6 day and 12 day-old rats, hearts from several animals were pooled to give 120-200 mg tissue for use in each binding assay. 120-200 mg from the apical half of ventricles of older animals were used. The tissues were thawed and homogenized in 25 volumes 250 mM sucrose, 10 mM Tris, 5 mM EDTA, 5 mM DTT, pH 7.5 with 3 bursts of 15 sec each of a mechanical tissue disruption device (Brinkman Polytron). The homogenates were centrifuged 10 min at 500 g and the pellet discarded. The supernatant was centrifuged 20 min at 45000 g and the resulting pellet was washed twice by resuspension in 40 ml 50 mM Tris, 10 mM $MgCl_2$, pH 7.5 and centrifugation for 20 min at 45000 g. The final pellet was suspended in this latter buffer at a protein concentration of .1 to .3 mg/ml.

Ventricular membranes were incubated in triplicate for 35 min at 25°C in an assay volume of 200 µl containing 50 mM Tris, 10 mM $MgCl_2$, and 60-600 pM [^{125}I]-HEAT (New England Nuclear) in the presence or absence of 10^{-6} M prazosin to define non-specific binding. Binding assays were terminated by filtration through Whatman GFC paper. Filters were washed 3 times with 4.5 ml incubation buffer at 0°C, and bound radioactivity determined by gamma ^{125}I counting at an efficiency of 60%. In pilot studies binding sites for [^{125}I]-HEAT identified under these conditions displayed the characteristics expected of $alpha_1$ adrenergic receptors.

Binding isotherms were analyzed by a non-linear least squares curve fitting procedure (4) to yield estimates of binding capacity and affinity for [^{125}I]-HEAT in each experiment. The mean values of such individually determined binding parameters from hearts derived from animals of different ages were then compared statistically by analysis of variance.

RESULTS

We observed a greater density of α_1AR (identified as specific binding sites for (^{125}I)-HEAT and expressed relative to membrane protein) in cardiac membranes of fetal, neonatal and weanling rats up to the age of 25 days than in mature rats ranging in age from 42 days to 24 months (Table I). This difference in receptor number between rats younger than 25 days and mature rats was highly significant ($p < .001$) and occurred in the absence of changes in receptor affinity.

Table 1
Alpha$_1$ adrenergic receptor characteristics in myocardial tissue from rats of varying age

Age (days)	Receptor density (fmol/mg protein)	Receptor affinity for IHEAT (nM)
fetal	108 ± 10*	.24 ± .13
1	91 ± 14	.231 ± .04
6	112 ± 14	.15 ± .03
12	98 ± 16	.18 ± .02
18	89 ± 12	.21 ± .04
25	80 ± 12	.23 ± .05
42	57 ± 2	.24 ± .05
90-120	54 ± 7	.23 ± .05
180	53 ± 7	.21 ± .06
700	50 ± 5	.23 ± .04

* mean of 5 or more animals ± SEM

Analysis of variance across all of the age groups confirmed a significant main effect of age upon receptor density ($p < .01$). Scheffe contrasts also revealed significant differences between each individual group up to 25 days and the pooled groups 42 days and older ($p < .01$). However, the trend towards

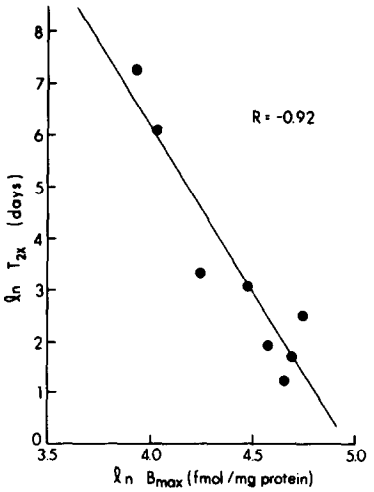

Figure 1. Natural log of cardiac growth rate, measured as the time in days required to double ventricular mass (ln T_{2x}), expressed as a function of the natural log of alpha$_1$ adrenergic receptor density (fmol/mg protein) in myocardial homogenates measured as Bmax for binding of IHEAT (ln Bmax).

fewer α_1AR when fetal and 1 day rats were compared to animals between 6 and 25 days of age was not statistically significant.

An inverse correlation ($r = .92$, $p < .01$) was present between the natural logs of the average receptor number in two consecutive age groups and the cardiac growth rate, expressed as doubling rate for ventricular weight (Fig. 1).

DISCUSSION

The present study describes the expression of α_1AR in membranes isolated from the ventricular myocardium of rats from fetal life to senescence. There appear to be two phases of α_1AR expression with respect to age: a relatively high concentration that is maintained from late fetal life through weaning (25 days); and a relatively lower concentration that is maintained from early adult life (42 days) throgh senescence (24 months).

A decrease in αAR concentration in the myocardium between weaning and adult stages has been previously reported in rats (5) and mice (6) and between young adult and senescent rats (7). However, the present study differs from these earlier reports in two respects: we did not observe lower α_1AR concentrations in the ventricular myocardium of fetal and newborn animals relative to immature rats, and we did not observe lower α_1AR concentrations in senescent rats relative to mature but younger animals. The basis of these discrepancies remains unresolved at this time.

These data support the viewpoint that α_1AR are expressed more fully in cardiac myocytes of the rat during periods of rapid cardiac growth. These data lend indirect support, therefore, to the hypothesis that α_1AR may exert a growth promoting function in the mammalian heart. The concept of a growth promoting role of cardiac α_1AR is enhanced by the recent evidence that α_1AR share common pathways of action with peptide hormones with more familiar growth promoting effects by stimulating membrane phosphotidyl inositol metabolism, release of diacylglycerol, and activation of protein kinase C (8). Further investigations are required to determine the physiological role of

α_1AR-mediated intracellular events in promoting cardiac growth during fetal and neonatal growth and in pathological hypertrophic states.

The responsiveness of ventricular strips to α_1 adrenergic stimulation varies with age (9), with 2 week old rat hearts showing much greater inotropic responses than hearts from 3 month and 26 month old rats. Therefore, in conjunction with the data from our current study, these findings suggest that physiologic responsiveness to α_1 adrenergic stimulation varies directly with α_1AR density in the sarcolemma.

REFERENCES

1. Williams, R.S., and Lefkowitz, R.J. (1978) Circ. Res. 43, 721-727.
2. Endoh, M.J., and Schumann, H.J. (1975) Nauyn-Schmiedebergs, Arch. Pharmacol. 287, 377-385.
3. Simpson, P. (1983) J. Clin. Invest. 72, 732-738.
4. Kent, R.S., DeLean, A., and Lefkowitz, R.J. (1980) Mol. Pharmacol. 17, 14-23.
5. Noguchi, A., and Whitselt, J.A. (1983) Eur. J. Pharmacol. 86, 43-50.
6. Yamada, S., Yamamura, H.I., and Roeske, W.R. (1980) Eur. J. Pharmacol. 68, 217-221.
7. Partilla, J.S., Hoopes, M.T., Ito, H., Dak, E.M., and Roth, G.S. (1982) Life Sci. 31, 2507-2512.
8. Berridge, M.J. (1984) Biochem. J. 220, 345-360.
9. Mackenzie, E., and Standen, N.B. (1980) Pflugers Arch. 383, 185-187.