BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Sep; 16 (5): 452—454

Alterations of subtypes of cardiac adrenoceptors in old rat[1]

YU Geng-Sheng, CHEN Ming-Zhe, HAN Qi-De[2] (*Institute of Vascular Medicine, Third Hospital, Beijing Medical University, Beijing 100083, China*)

AIM: To determine alterations of subtypes of myocardial adrenoceptors in senescence. **METHODS**: Heart membrane preparations were made from 3- and 25-month old Wistar rats. α_1- and β-adrenoceptors were measured by radioligand, ^{125}I-BE2254 and ^{125}I-pindolol, binding assays, respectively. **RESULTS**: In the old rat heart, α_1- and β-adrenoceptor densities were declined from the young rats of 119±4 and 45.9±1.9 pmol L^{-1} to 70 ±6 and 36.4±1.6 pmol L^{-1} ($P<0.01$), with a greater change in α_1-AR and in β-AR. The ratio of α_{1A}/α_{1B} subtypes was decreased from the young rats of 39/61 to the old rats of 26/74 ($P<0.05$). **CONCLUSION**: The cardiac adrenoceptors are decreased with different extents in the different subtypes in old rats.

KEY WORDS aging; alpha-1 adrenergic receptors; beta adrenergic receptors; heart; radioligand assay

In senescence, positive inotropic and chronotropic responses induced by catecholamine were diminished in mammalian hearts[1,2]. That the density of α_1-adrenoceptors in old rat heart decreased significantly, as compared with the young rats, may be, at least in part, the reason for the decreased adrenergic responsiveness in the heart[1]. The alteration of β-adrenoceptors was not only at the receptor level but also in the coupling to the adenylate cyclase via G-proteins[3], while the density of β-adrenoceptors decreased in the old rat heart[4].

The change of subtypes of cardiac adrenoceptors in senescence is not clear. We have reported that the ratio of α_{1A}/α_{1B} subtype elevated significantly in the blood vessels of old rats[5]. But in parotid cells age does not alter the ratio[6]. It suggests that the alterations of α_1-adrenoceptor subtypes in different tissues would be expressed in different manner. The purpose of the present work is to determine the alterations of cardiac α_1-adrenoceptor subtypes and β-adrenoceptors in the old rats.

MATERIALS AND METHODS

Drugs BE2254 (2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone (Beiersdorf); chloroethylclonidine (CEC), *dl*-propranolol, phentolamine; (−)-pindolol (Sigma); carrier-free $Na^{125}I$ (Institute of Atomic Energy, Chinese Academy of Sciences).

Tissue preparation for radioligand binding Wistar rats, 3 or 25 months old, were killed by cervical dislocation. The hearts were homogenized in cold phosphorate 20 mmol L^{-1} buffer solution (PBS, pH 7.6). After centrifuged at 20 000 × *g* at 4 C for 10 min, the pellets were made to the appropriate tissue concentration.

CEC pretreatment Aliquots (usually 10 mL) of the resuspended preparation were incubated at 37 C with or without CEC (20 μmol L^{-1}) in HEPES buffer (pH 7.6) for 10 min. Reactions were stopped by adding 20 mL cold PBS, centrifuged at 20 000 × *g* for 10 min. The pellets were washed with cold PBS twice and resuspended in 10 mL PBS.

Radioligand binding BE2254 (BE) and (−)-pindolol (Pin) were radioiodinated to specific activity of 81.4 PBq mol^{-1} and stored at −20 C in methanol. Measurement of specific ^{125}I-BE or ^{125}I-PIN binding

[1] Project supported by the Research Grant from Ministry of Public Health of China, № 91103015, from the National Natural Science Foundation of China, № 39370299, № 39470806, and from China Medical Board of New York, № 93—591

[2] Correspondence to Prof HAN Qi-De.

Received 1994-08-08 Accepted 1995-02-24

were performed by incubating 0.1 mL tissue preparations with ^{125}I-BE or ^{125}I-Pin in PBS (final volume 0.25 mL) at 37 C in the presence or absence of competing drugs for 20 min. The incubation was terminated by adding Tris-HCl (10 mmol L^{-1}, pH 7.4) 10 mL and filtering over a glass fiber filter (Schleicher and Schuell, No 30. Keene NH. USA) under vacuum. Each filter was washed with 10 mL of Tris-HCl (10 mmol L^{-1}) buffer and dried; then the radioactivity was measured. Nonreceptor binding was determined to be binding in the presence of phentolamine (10 μmol L^{-1}) or propranolol (10 μmol L^{-1}). Saturation was determined by incubating tissue with increasing concentrations of ^{125}I-BE (25 — 500 pmol L^{-1}) or ^{125}I-Pin (50 — 1000 pmol L^{-1}) and the data were analyzed by the method of Scatchard.

Statistics All the data were expressed as $\bar{x} \pm s$, and t-test was used to compare the difference.

RESULTS

The specific binding sites of ^{125}I-BE (B_{max}) in the preparations from old rat hearts were decreased while the dissociation constant (K_D) was increased significantly ($P<0.01$), as compared with the young rats. After the incubation with CEC 20 μmol L^{-1} for 10 min at 37 C, the B_{max} was decreased by 74±8 % in the old rats, but decreased by only 61 ± 5 % ($P<0.05$) in the young rats (Tab 1).

Tab 1. ^{125}I-BE and specific binding sites in hearts of young and old rats. $\bar{x} \pm s$.
$^{a}P>0.05$, $^{c}P<0.01$ *vs* young.

Rats	Young ($n=6$)	Old ($n=5$)
Control		
K_D	29±4	57±4^{c}
B_{max}	119±4	70±6^{c}
CEC pretreated		
K_D	59±10	63.5±1.3^{a}
B_{max}	46±3	18.1±1.2^{c}

K_D: pmol L^{-1}; B_{max}: pmol/g protein

In the old rats ($n=5$), the densities of β-adrenoceptors (B_{max}) were decreased from the young rats ($n=6$) of 45.9±1.9 pmol/g protein to 36.4±1.6 pmol/g protein ($P<0.01$). But there was no change in K_D values as compared with the young rats (83±10 *vs* 68±5 pmol L^{-1}, $P>0.05$). The loss of β-adrenoceptors in old rat heart was less severe than that of α_1-adrenoceptors as the ratio of β- and α_1-adrenoceptors was increased from the young rats of 39.5 ± 1.7 % to 53 ± 4 % ($P<0.05$).

DISCUSSION

Cardiovascular impairment is one of the most significant functional manifestations of the aging and lots of evidence suggest that myocardial adrenergic responsiveness is reduced in senescence[1-4]. The results of this study showed that the density of β-adrenoceptors in old rat heart was decreased significantly as compared with the young rats, whereas the K_D value without any difference between the 2 groups. It implied that the decline of cardiac β-adrenoceptors might be a reason for the diminished response of myocardiac tissue to catecholamine in the senescence.

It was reported that α_{1A}-subtype might be involved in the induction of embryonic gene expression in ventricular cell hypertrophy whereas α_{1b}-subtype medicated positive inotropic effects in rat heart[7,8]. In our experiments, we found that the myocardial α_1-adrenoceptors were decreased in density and increased in K_D value in the old rat, as compared with the young rats. Pre-treatment of the preparations with CEC 20 μmol L^{-1} decreased the ^{125}I-BE binding sites by 74 % in old rat and 61 % in the young rats which suggested that the proportion of myocardial α_{1B}-subtype was increased significantly, but in contrast to the results of functional experiments in blood vessels[5]. From these results we might be able to speculate that the α_{1B}-sub-

type would take an increasingly important role in cardiac inotropic response mediated by α_1-adrenoceptors and the changes of α_1-adrenoceptor subtypes would express tissue specifically in the rat with aging, although the significance of different α_1-adrenoceptor subtypes in rat tissues still need to be further studied. In addition, our results disagree with that of Gascon *et al*[9]. What created the discrepancy was not clear, but the concentration of CEC used in the experiments might be a critical factor that would be considered seriously.

Interestingly, in present experiments, the ratio of β/α_1 adrenoceptors was increased in the old rat heart, which suggested that the decline of α_1-adrenoceptors was greater than that of β-adrenoceptors. However, what about the effects of this alteration in the ratio of α_1- and β-adrenoceptors on the function of the heart from old rat is not clear. Since both α_1- and β-adrenoceptors are involved in the regulation of heart function, their relative role in the inotropic effect, induce by catecholamine, of old rat heart must be further conducted.

REFERENCES

1 Kimball KA, Cornett LE, Seifen E, Kennedy RH. Aging: changes in cardiac α_1-adrenoceptor responsiveness and expression. Eur J Pharmacol-Mol Pharmacol Sec 1991; **208**: 231—8.

2 Docherty JR. Changes in adrenoceptor function with age. In: Bevan JA, Majewski H, Maxwell RA, Story DF, editors. Vascular neuroeffector mechanisms. Oxford, WA: IRL Press, 1988; 281—7.

3 Robberecht P, Gillard M, Waelbroeck M, Camus JC, De Neef P, Christophe J. Alterations of rat cardiac adenylate cyclase activity with age. Eur J Pharmacol 1986; **126**: 91—5.

4 Fan T-HM, Banerjee SP. Age-related reduction of beta-adrenoceptor sensitivity in rat heart occurs by multiple mechanisms. Gerontology 1985; **31**: 373—80.

5 Han QD, Li JL. Changes of the α_1-adrenoceptor and its subtypes in blood vessels of old rats. Acta Physiol Sin 1991; **43**: 22—30.

6 Villalobos-Molina R, Miyamoto A, Kowatch MA, Roth GS. α_1-Adrenoceptors in parotid cells: age does not alter the ratio of α_{1A} and α_{1B} subtypes. Eur J Pharmacol-Mol Pharmacol Sec 1992; **226**: 129—31.

7 Michel MC, Knowlton KU, Gross G, Chien KP. α_1-Adrenergic receptor subtypes mediated distinct functions in adult and neonatal rat heart (Abstract). Circulation 1990; **82** Suppl 4: III—561.

8 Simpson PC, Cuenco RG, Paninghatan MO, Murphy MD. An α_1-adrenergic receptor subtypes sensitive to WB4101 transduces cardiac myocyte growth (Abstract). Circulation 1990; **82** Suppl 4: III—561.

9 Gascon S, Dierssen M, Marmol F, Vivas NM, Badia A. Effects of age on α_1-adrenoceptor subtypes in the heart ventricular muscle of the rat. J Pharm Pharmacol 1993; **45**: 907—9.

452—454

18

老年大鼠心脏肾上腺素受体亚型的改变

禹更生，陈明哲，韩启德 （北京医科大学第三医院血管医学研究所，北京100083，中国）

R337.38

目的：研究老年心脏肾上腺素受体亚型的改变. **方法**：制备3月龄与25月龄 Wistar 大鼠心脏膜标本，分别采用^{125}I-BE2254与^{125}I-Pindold作放射配基结合分析，测定 α_1与 β-肾上腺素受体. **结果**：老年大鼠心脏的 α_1与 β-肾上腺受体密度分别由年轻大鼠的119±4与45.9±1.9 pmol/g 下降至70±6与36.4±1.6 pmol/g ($P<0.01$)，α_1-AR 降低的幅度大于β-AR. 此外 α_{1A}与 α_{1B}亚型之比由年轻大鼠的39:61下降至26:74 ($P<0.05$). **结论**：老年大鼠心脏肾上腺素受体减少，且不同亚型减少的程度不同.

关键词 衰老；α肾上腺素受体；β肾上腺素受体；心脏；放射配位体测定