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Altered α_1-adrenoceptor density and α_{1A}/α_{1B} ratio in rat hearts after propranolol treatment[1]

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ABSTRACT After rats were treated with propranolol (50 mg·kg^{-1}, bid, ip) for 7 d, the density of α_1-adrenoceptors in the rat myocardial cell membranes increased from 137±25 to 178±30 fmol/mg protein determined by receptor radioligand assay. Whereas the K_D value was not significantly changed, the relative proportion of α_{1A}-adrenoceptor subtype increased from 19±6 to 31±8 %. The affinities of two subtypes to 5-methylurapidil were not obviously changed after propranolol treatment. It was suggested that α_{1A}-subtype was more important in α_1-receptor mediated alterations after β-adrenoceptor blockade.

KEY WORDS alpha adrenergic receptors; radioligand assay; propranolol; 5-methylurapidil; myocardium; cell membrane; prazosin

Adrenergic α_1- and β-receptors coexist in hearts of various species. Both receptor systems mediate positive inotropic effects through different second messengers. However, β-adrenoceptors play the most important role in regulating heart rate and contraction force. When β-adrenoceptors were blocked, α_1-receptors might compensate for above cardiac functions[1,2]. Studies have implied the increase of α_1-receptor density in rat hearts after chronic treatment with propranolol [3,4] Alpha$_1$-adrenoceptors are mainly composed of two subtypes, α_{1A} and α_{1B}. The present study aimed to examine the alteration of α_1-receptor subtypes in rat hearts after β-receptors were blocked by propranolol chronically.

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MATERIALS AND METHODS

Wistar rats (235±*s* 13 g) were treated with propranolol (50 mg·kg^{-1}, bid, ip) or 0.9 % saline (as control) for 7 d.

Preparation of heart membrane Rats were decapitated. Ventricles were isolated, chopped, and homogenized twice for 10 s with a Polytron in 7 ml ice-cold buffer (Tris-HCl 50, NaCl 100, edetic acid 2 mmol·L^{-1}), pH 7.4. The homogenate was centrifuged at 350×*g* for 10 min. The supernatant was centrifuged at 45 000×*g* for 20 min. The pellets were resuspended and washed twice in fresh buffer (Tris-HCl 50, edetic acid 1 mmol·L^{-1}), pH 7.4. The final pellets were resuspended and homogenized in ice-cold buffer again, and filtered through two layers of gauze.

Receptor binding studies Saturation binding analysis was performed with 6 concentrations of [^{3}H]prazosin 25—400 pmol·L^{-1}. Membrane preparations (500 μl) were incubated with [^{3}H]prazosin in a final volume of 1 ml at 30 ℃ for 45 min. Incubations were carried out in duplicate. Parallel incubations were conducted in the presence of phentolamine (100 μmol·L^{-1}) to determine nonspecific binding. Incubations were termined by rapid vaccum filtration and two washes with 5 ml ice-cold buffer. Dried filters were counted in scintillation liquid with a Beckmann LS 9800 scintillation counter. K_D values and maximal binding (B_{max}) were calculated from Scatchard plot.

Competition analysis was performed with 16 concentrations of 5-methylurapidil (5-MU) ranged from 50 pmol·L^{-1} to 50 μmol·L^{-1}. Curves were fitted by nonlinear least-square technic. The concentrations of 5-MU necessary to inhibite half of the specific [^{3}H]prazosin binding (IC_{50} value) at 2 α_1-subtypes and

the ratio of receptor subtypes were calculated. The K_i value was calculated by the following formula:

$$K_i = IC_{50}/(1+[L]/K_D)$$

where [L] represents the concentration of [^{3}H]prazosin.

Drugs *dl*-Propranolol and phentolamine (Sigma), [^{3}H]prazosin (9.25 GBq·mol^{-1}, Du Pont NEN Research Products), 5-MU was produced by Byk Gulden (Konstanz, F R Germany).

Statistics Values of $\bar{x}\pm s$ were compared with t test for unpaired observations.

RESULTS

Alpha$_1$-adrenoceptor density Body weights and heart weights between control (232±s 11 g, 0.9±s 0.1 g) and propranolol treated rats (237±s 10 g, 0.9±s 0.1 g) had no obvious differences. Binding capacity of [^{3}H]prazosin with α_1-receptors in ventricular membrane preparation was higher in propranolol-treated rats (Fig 1).

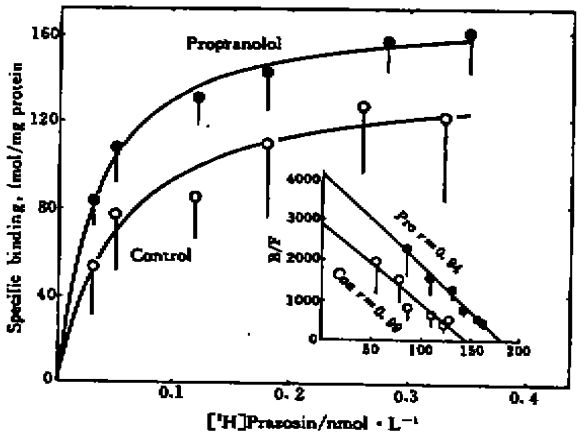

Fig 1. Specific binding of [^{3}H]prazosin to cardiac membranes in control (n=6) and propranolol-treated (n=7) rats. $\bar{x}\pm s$. Inset, Scatchard plot.

Scatchard analysis showed single component for both groups. The receptor density increased from 137±s 25 fmol/mg protein (n = 6) in control to 178±s 30 fmol/mg protein (n = 7) in propranolol treated rats. However, the K_D values had no significant differences between the 2 groups (63±31 *vs* 72 ±44 pmol·L^{-1}).

Alpha$_1$-adrenoceptor subtypes Displacement of [^{3}H]prazosin binding with sixteen incremental concentrations of 5-MU produced a biphasic competition curve (Fig 2).

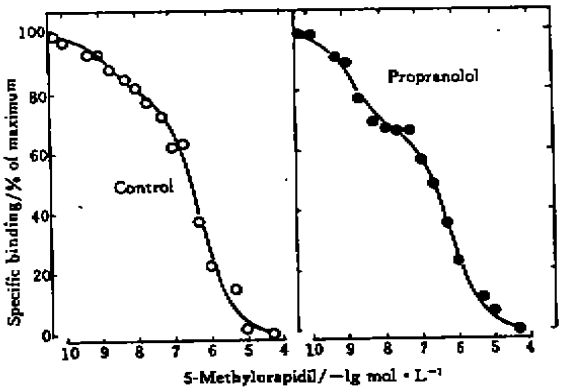

Fig 2. Inhibition (%) of [^{3}H]prazosin binding to cardiac membranes by 5-methylurapidil in control and propranolol treated rat hearts.

The Hill coefficent calculated from 13 experiments was 0.57±0.09, indicating 2 different receptor binding sites to 5-MU. pK_i values of 5-MU to inhibite [^{3}H]prazosin binding at α_{1A} and α_{1B} were calculated from IC_{50} values by nonlinear regression. In control and test group pK_i values for α_{1A} subtype were 9.5 ±0.7 and 9.2±1.0, and for α_{1B} subtype 6.8 ± 0.4 and 6.9 ± 0.4, respectively. There were no significant differences between the 2 groups, whereas the relative proportion of α_{1A}-adrenoceptors increased from 19 ± 6 in control to 31±8 % in test group ($P<0.05$).

DISCUSSION

The present study showed the density of α_1-adrenoceptors increased after chronic treatment with proprenolol. This increase of α_1-adrenoceptor density would be a compensation when the physiological function of β-

adrenoceptors were depressed[16-17].

It has been known in displacement that the component showing high affinity to 5-MU represents α_{1A} binding sites and the component with low affinity to 5-MU belongs to α_{1B} receptor sites. In this study we found that the proportion of α_{1A} significantly increased after treatment with propranolol. But the pK_i values for 5-MU to inhibit both subtypes, which were similar to the data of Gross *et al*[15], were not obviously changed, indicating that the α_1-adrenoceptor density increase after propranolol treatment was mainly due to the increase of α_{1A} subtype.

Our previous studies[19] showed that the regulation of cardiac α_{1A} subtype is more sensitive to pathological condition and drugs compared with α_{1B} subtype, implying that α_{1A}-subtype was a more potently compensatory factor for physiological alteration, especially in the state that β-adrenoceptor activity was depressed.

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普萘洛尔对大鼠心肌细胞膜 α_1 肾上腺素受体密度及 α_{1A}/α_{1B} 比例的影响

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A **摘要** 放射配基结合实验表明, 大鼠 ip 普萘洛尔(Pro) 7 d 后, 心肌细胞膜 α_1 肾上腺素能受体密度由137±25增加到 178±30 fmol/mg protein ($P<0.05$), K_D 值无显著改变. 5-MU 竞争结合实验表明, 使用 Pro 后 α_{1A} 亚型在 α_1 受体总数中所占比例由19±6 % 增加到31±8 % ($P<0.05$). 但两种受体亚型的亲和力都未改变. 说明 β 受体阻断后, α_{1A} 亚型变化更为敏感.

关键词 α 肾上腺素能受体; 放射配位体测定; 普萘洛尔; 心肌; 细胞膜; 哌唑嗪; 5-甲基乌拉地尔