BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Jul; 14 (4) : 298—301

Determination of β-adrenoceptor subtypes in human pulmonary artery and thoracic aorta by radioligand binding

PENG Hong-Li, JIANG Ming-Hua, YANG Zao-Chen[1]
(*Department of Pharmacology, School of Pharmacy; [1] Department of Pharmacology, Faculty of Basic Medical Sciences, Shanghai Medical University, Shanghai 200032, China*)

ABSTRACT The β-adrenoceptors of human pulmonary artery (PA) and thoracic aorta (TA) were studied by the use of a high specific activity radioligand [^{125}I]pindolol (Pin). To identify the subtypes of β-adrenoceptors in the 2 blood vessels, the competitive inhibition curves of [^{125}I]Pin by β_1-antagonist atenolol and β_2-agonist salbutamol were analyzed using a computer program LIGAND of a mathematical model of the ligand-binding system. The B_{max} (15.3 ± 1.2 fmol/mg protein) and the dissociation constant K_d (44±4 pmol·L^{-1}) for PA were similar to those for TA (B_{max}, 12.8 ± 1.2 fmol/mg protein, K_d, 45 ± 4 pmol·L^{-1}). Competitive inhibition analysis showed that the β_1-and β_2-adrenoceptor subtypes coexisted in human PA and TA, with β_1 adrenoceptor dominant. The ratios of β_1 : β_2 in PA and TA were 2.9 : 1.0 and 2.1 : 1.0, respectively.

KEY WORDS beta adrenergic receptors; pulmonary artery; thoracic aorta; radioligand assay; pindolol; atenolol; salbutamol

β-Adrenoceptors were classified into β_1- and β_2-receptors (Lands *et al.* 1967). Both β_1- and β_2-receptors may coexist within the same tissue such as myocardium (β_1 predominant), lung and bronchus (β_2 predominant)[1]. Rat pulmonary artery, aorta and portal vein contained both β_1- and β_2- adrenoceptors[2,3]. But autoradiographic analysis in guinea pig and human lung indicated that the receptors in pulmonary arteries were of β_2 subtypes[4,5]. Few studies have been carried out to determine the distribution of β-adrenoceptor subtypes in the human vascular bed. This study was aimed to identify and characterize β-adrenoceptor in human pulmonary artery and thoracic aorta, using the high specific radioligand [^{125}I]pindolol (Pin) and atenolol (as β_1 antagonist) and salbutamol (as β_2 agonist).

Received 1992-04-16 Accepted 1993-02-26
[1] Project supported by the National Natural Science Foundation of China, № 3870881.
[2] This paper was included in the Proceedings of the 5th National Neuropharmacology Conference, 1992 Mar 15—18, Shanghai, China.

MATERIALS AND METHODS

Membrane preparation Human main pulmonary arteries (PA) and thoracic aorta (TA) were taken during 4 autopsies (25±5 a) within 2 h after accidental death. Membranes from the vessels were prepared[6]. The vessels were trimmed of adventitia in buffer A (sucrose 0.25 mol·L^{-1}, Tris 10, $MgCl_2$ 1 mmol·L^{-1}, pH 7.4), and homogenized thrice using a ZS 83-1 tissue homogenizer for 30 s. The homogenate was centrifuged (3000×g) for 10 min. The supernatant was centrifuged at 37 000×g for 20 min and resuspended in buffer B (Tris 20, $MgCl_2$ 1, EDTA 1, ascorbic acid 1.1 mmol·L^{-1}, pH 7.4). The experiments were carried out at 4℃. The membranes were stored at −20℃. Protein concentration was determined by Coomassie brilliant blue. [^{125}I]Pin was purchased from Shanghai Second Medical University (70.30 TBq).

β-Adrenoceptor binding studies Human PA and TA membrane suspension 100 μg were incubated in a water bath at 37℃ for 30 min in the presence of [^{125}I]Pin escalating (25—250 pmol·L^{-1}) with or without unlabeled propranolol (0.03 mmol·L^{-1}). The final volume was 400 μl. After incubation, the mixture was immediately filtered at 4℃, trapping the membrane particles on 2T-II filters, which were washed with 18 ml buffer C (Tris 20, EDTA 1, $MgCl_2$ 10 mmol·L^{-1}, pH 7.4) for 20 s. The radioactivity of the filter was measured in a LKB COMPUGAMA 1282 counter.

Proportion of β-adrenoceptor subtypes By a

quantitative analysis[7], these subtypes in the plasma memrane homogenates of human PA and TA were determined by a competitive inhibition study between [^{125}I]Pin (200 pmol·L^{-1}) and the selective β_1-antagonist atenolol (10^{-9} to 10^{-3} mol·L^{-1}) or the selective β_2-agonist salbutamol (10^{-9} to 10^{-3} mol·L^{-1}). The reaction tubes contained plasma membrane homogenate 100 μg (200 μl), 50 μl of [^{125}I]Pin (200 pmol·L^{-1}), and 50 μl of atenolol or salbutamol. The incubation was carried out same as that in the saturation binding experiments. Specific binding of the ligand was defined as the amount of the labels bound in the absence of competing ligand minus the amount bound in the presence of propranolol (0.03 mmol·L^{-1}).

Analysis of data The data given in the paper were $\bar{x} \pm s$. The dissociation constant (K_d) and the maximal number of binding sites (B_{max}) were calculated from Scathard and Hill plots. For the identification of the vessel receptors, the concentration-inhibition curves were transformed into modified Scatchard plots by plotting the % inhibition of binding *vs* % inhibition divided by the concentration of atenolol or salbutamol. The competitive curves were analyzed with a computer model[8].

RESULTS

[^{125}I]Pin binding to human PA and TA Specific [^{125}I]Pin binding elevated with escalating concentrations of [^{125}I]Pin from 25 to 250 pmol·L^{-1}. Scatchard analysis (Fig 1) gave linear plots and the Hill coefficients were both 1. The receptor density (B_{max}, 15.3 ±1.2 fmol/mg protein) and the dissociation constant (K_d, 44±4 pmol·L^{-1}) for PA were similar to those for TA (B_{max}, 12.8±1.2 fmol/mg protein, K_d, 45±4 pmol·L^{-1}).

Inhibition of [^{125}I]Pin binding by atenolol and salbutamol The transformed Scatchard plots of the inhibition curves of [^{125}I]Pin binding by atenolol and salbutamol were shown in Fig 2. Computer modeling of these curves according to a 2-class model yielded the % distribution of β_1 *vs* β_2-adrenoceptors (Tab 1). The % distributions were 78:22 (PA), 69:31 (TA) with atenolol and 69:31 (PA), 68:32 (TA) with salbutamol.

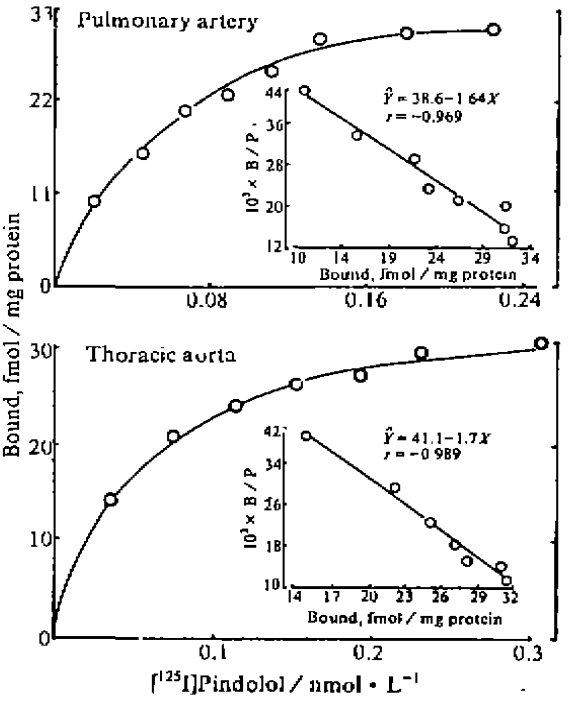

Fig 1. Specific binding of [^{125}I]Pin to membranes from human pulmonary artery and thoracic aorta. Inset, Scatchard plots.

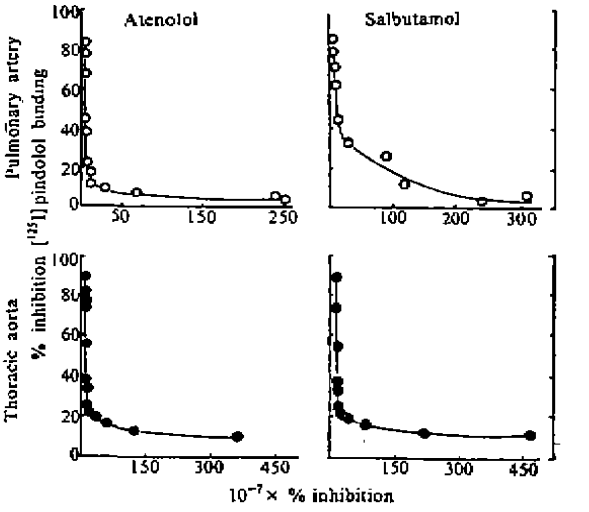

Fig 2. Scatchard plots of inhibition bindings between [^{125}I]Pin and atenolol or salbutamol to human pulmonary artery and thoracic aorta membranes.

Tab 1. Competitive inhibition binding with [^{125}I]Pin and atenolol or salbutamol, dissociation constants for atenolol and salbutamol, percentage of β_1- and β_2-adrenoceptors binding sites in human pulmonary artery and thoracic aorta membranes in 3 men. $\bar{x}\pm s$.

	Pulmonary artery	Thoracic aorta
Atenolol K_d/mol·L^{-1}		
β_1	$(4.5\pm0.9)\times10^{-8}$	$(1.1\pm0.3)\times10^{-8}$
β_2	$(4.4\pm0.5)\times10^{-6}$	$(2.6\pm1.3)\times10^{-6}$
Subtypes		
β_1 : β_2/%	78±5 : 22±5	69±11 : 31±11
Salbutamol K_d/mol·L^{-1}		
β_1	$(4.1\pm2.8)\times10^{-6}$	$(2.6\pm0.8)\times10^{-6}$
β_2	$(4.9\pm1.8)\times10^{-8}$	$(4.5\pm1.6)\times10^{-8}$
Subtypes		
β_1 : β_2/%	69±7:31±7	68±2:32±2

DISCUSSION

Although vasodilatation in most of blood vessels is considered to be mediated by β_2-receptor subtype, the receptor characteristics in large coronary artery were shown to be β_1-subtype predominant[9]. These data were obtained mainly from swine, bovines, canines, felines, lapins, etc., and from Japanese monkeys[10]. Receptor subtypes mediating the vascular response frequently differ in primates from lower mammals[11]. Grigorian *et al*[12] found that the properties of β-adrenoceptor in the membranes of cultured endothelial cells from the human pulmonary artery and umbilical vein showed no obvious difference. But they did not quantitate the subtypes in the vessels. In this study, β-adrenoceptor binding characteristics and subtypes were primarily determined in human pulmonary artery and throacic aorta. Binding of [^{125}I]Pin to pulmonary artery and throacic aorta membranes exhibited the pharmacologic characteristics expected for labeling of β-adrenoceptor. Equilibrium was reached during incubation, saturation was attained, specific binding was of high affinity and cooperativity was not evident. Scatchard analysis of the specific binding data indicated that the binding of radio-ligand was to a single population of receptors. Selective β_1- antagonist (atenolol) and β_2- agonist (salbutamol) were used to quanititate the subtypes of β-adrenoceptors in the human main pulmonary artery and throacic aorta. Nonlinear Scatchard plots suggeted the presence of two binding sites, one of the high affinity and the other, of low affinity. For atenolol, the high affinity component was related to β_1-adrenoceptors, while for salbutamol, to β_2-adrenoceptors. It was concluded that both β-adrenoceptor subtypes coexisted in the 2 vessels and the β_1-receptor subtype was predominant.

[^{125}I]Pin is an ideal ligand for this kind of study. It binds to the β_1- and β_2-adrenoceptors with equally high affinity and has a very low non-specific binding. With atenolol, the proportions of high affinity sites (β_1) were 78% (PA), 69% (TA), salbutamol produced an exact "mirror image", the proportions of high affinity sites (β_2) being 31% (PA), 32% (TA). The use of 2 different agents with different affinity and selectivities yeilded essentisllly constant percentage of β_1- and β_2-adrenoceptors. In summary, this paper disclosed the feature of the distribution of β-adrenoceptor subtypes in human pulmonary artery and thoraic aorta.

REFERENCES

1 Brown L, Deighton NM, Bala S, Söhlmann W, Zerkowski HR, Michel MC, *et al*. Spare receptors for β-adrenoceptor-mediated positive inotropic effects of catecholamines in the human heart.
J Cardiovasc Pharmacol 1992; **19**: 222—32.

2 Chin JPF, Pennefather JN. Classification of the β-adrenoceptor subtype in the rat portal vein: effect of altered thyroid hormone levels.
Eur J Pharmacol 1992; **212**: 201—7.

3 O'Donnell SR, Wanstall JC. Beta-1 and beta-2 adreno-

ceptor-mediated responses in preparations of pulmonary artery and aorta from young and aged rats. *J Pharmacol Exp Ther* 1984; **228**: 733−8.

4 Gatto C, Johnson MG, Seybold V, Kulik TJ, Lock JE, Johnson DE. Distribution and quantitative developmental changes in guinea pig pulmonary β-receptors. *J Appl Physiol* 1984; **57**: 1901−7.

5 Carstairs JR, Nimmo AJ, Barnes PJ. Autoradiographic visualization of beta-adrenoceptor subtypes in human lung. *Am Rev Respir Dis* 1985; **132**: 541−7.

6 Schwartz J, Velly J. The β-adrenoceptor of pig coronary arteries: determination of β_1 and β_2 subtypes by radioligand binding. *Br J Pharmacol* 1983; **79**: 409−14.

7 Molinoff Pb, Wolf BB, Weiland GA. Quantitative analysis of drug-receptor interactions: II Determination of the properties of receptor subtypes. *Life Sci* 1981; **29**: 427−43.

8 Munson PJ, Rodband D. Ligand: A versatile computerized approach for characterization of ligand-binding systems. *Anal Biochem* 1980; **107**: 220−39.

9 Young MA, Knight DR, Vatner SF. Autonomic control of large coronary arteries and resistance vessels. *Prog Cardiovasc Dis* 1987; **30**: 211−34.

10 Toda N, Okamura T. Beta adrenoceptor subtype in isolated human, monkey and dog epicardial coronary arteries. *J Pharmacol Exp Ther* 1990; **253**: 518−24.

11 Toda N. Alpha adrenergic receptor subtypes in human, monkey and dog cerebral arteries. *J Pharmacol Exp Ther* 1983; **226**: 861−8.

12 Grigorian G, Mirzapoyazova AV, Nikashin AV, Goncharov NA, Danilov SM. Identification and characterization of β-adrenoreceptors in the membranes of cultured endothelial cells from the human pulmonary artery. *Probl Endocrinol* (Mosk) 1989; **35** (4): 37−40.

298-301 (4)

放射配体结合分析法检测人肺动脉和胸主动脉β肾上腺素受体的亚型

彭红丽，江明华，杨藻宸[1] R965.2

(上海医科大学药学院药理教研室，[1]上海医科大学基础医学部药理教研室，上海200032，中国)

摘要 用β_1受体阻滞剂阿替洛尔和β_2受体激动剂沙丁胺醇与[^{125}I]pindolol对人肺动脉和主动脉膜上的β_1和β_2受体进行竞争性抑制反应，基于配基-高分子相互作用原理用LIGAND软件分析竞争结合数据. 结果表明肺动脉和胸主动脉上β_1和β_2受体是共存的，且以β_1受体占优势，其β_1：β_2的比率为2.9：1.0（肺动脉），2.1：1.0（胸主动脉）.

肾上腺素受体 测定

关键词 β肾上腺素能受体；肺动脉；胸主动脉；放射配体结合测定；阿替洛尔；沙丁胺醇；吲哚洛尔

BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Jul; 14 (4): 301−305

Antifibrillatory effect of tetrahydroberberine

SUN An-Yang, LI De-Xing

(*Department of Pharmacology, Nanjing Medical College, Nanjing 210029, China*)

ABSTRACT Electric stimulation and drug-induced ventricular fibrillation (VF), monopbasic action potentials (MAP), and triggered activity were studied before and after administration of tetrahydroberberine (THB) in rabbits, rats or guinea pigs. At doses of 5, 10, and 20 $mg \cdot kg^{-1}$, iv THB increased the ventricular fibrillation threshold, and the $BaCl_2$-induced VF was also prevented or terminated by THB in rabbits. Centrogenic VF induced by icv aconitine in rats was inhibited by pretreatment with THB in a dose-dependent manner, whereas VF induced by iv ouabain in guinea pig was inhibited to a lesser degree. For MAP, the duration at 90% repolarization ($MAPD_{90}$) was prolonged remarkably, whereas the $MAPD_{50}$, the MAP amplitude, and the maximal velocity of phase 0 were shortened or decreased slightly. The amplitudes of early afterdepolarization produced by cesium chloride (CsCl) were attenuated, while the cumulative threshold doses of CsCl for sustained ventricular tachycardia were elevated by THB.

These results indicated that THB had an potent antifibrillatory effect, which might be attributed to its

Received 1992-03-30 Accepted 1993-02-15