BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Nov; 14 (6) : 492—495

A new α_1-adrenergic receptor subtype with low affinity for 5-methyl-urapidil but insensitive to chlorethylclonidine[1]

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

ABSTRACT In the present study, we found that a novel α_1-adrenergic receptor subtype existed in rat heart by radioligand binding assay. This new α_1-adrenergic receptor subtype was different from α_{1A}-, α_{1B}-, α_{1C}-, and α_{1D}-subtypes reported recently. It had high affinity for WB4101, as the same of α_{1A}-subtype, but low affinity for 5-methyl-urapidil, and was insensitive to chlorethylclonidine.

KEY WORDS alpha-adrenergic receptors; heart; WB4101; 5-methyl-urapidil; chlorethylclonidine

We have previously identified that the α_1-adrenergic receptor in rat heart could be subdivided into 2 subtypes, α_{1A}(1/3) and α_{1B}(2/3), according to their sensitivities to the alkylating agent chlorethylclonidine (CEC) and their affinities for the competitive antagonist WB4101[1]. It was considered that 5-methyl-urapidil bound to α_{1A}-subtype with high affinity in rat tissues, similar with WB4101[6]. The evidences of new α_1-adrenergic receptor subtypes elucidated by gene cloning raised the possibility of the existence of more than two α_1-adrenergic receptor subtypes[9].

In present study, we compared the characteristics in inhibition of 5-methyl-urapidil and WB4101 for ^{125}I-BE specific binding in pre- and pro-CEC-treated rat heart preparations, and attempt to determine whether an additional α_1-adrenergic receptor subtype could be distinguished by the methods of radioligand binding assay.

Received 1992-12-07 Accepted 1993-08-11

[1] Project supported by the Research Grant from Administration of Public Health of China, № 91103015.

MATERIALS AND METHODS

Materials 2-β (4-Hydroxyphenyl)-ethylaminomethyl)-tetralone (BE2254, Beiersdorf, Hamburg, Germany); chlorethylclonidine (CEC), 2-(2, 6-dimethoxyphenyl)-ehtyl)-aminomethyl-1, 4-benzodioxane (WB4101), 5-methyl-urapidil (Research Biochemicals Inc, Wayland MA, USA); Carrier-free Na^{125}I (China Atomic Research Institute).

Tissue preparation for radioligand binding Wistar rats (♂, 200 ± *s* 12 g) were killed by cervical dislocation. Hearts were homogenized in cold 20 mmol·L^{-1} phosphorate buffer (PBS, pH 7.6)[1]. After centrifuged at 20 000×*g*, 4℃ for 10 min, the pellets were specified to the appropriate tissue concentration.

CEC treatment Aliquots (usually 10 ml) of the resuspended preparation were incubated at 37℃ with or without CEC (10 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.

^{125}I-BE binding BE2254 was radioiodinated to theoretical specific activity (81.4 PBq·mol^{-1}) and stored at −20℃ in methanol[2]. Measurement of specific ^{125}I-BE binding was performed by incubating 0.1 ml tissue preparations with ^{125}I-BE in PBS (final volume 0.25 ml) at 37℃ in the presence or absence of competing drugs for 20 min. The incubation was terminated by adding 10 ml of Tris-HCl (10 mmol·L^{-1}, pH 7.4) and filtering over a glass fiber filter (Schleicher and Schuell № 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}).

Analysis of binding data Saturation curves were determined by incubating tissue with increasing concentrations of ^{125}I-BE (25 − 500 pmol·L^{-1}) and

analyzing the data by the method of Scatchard[3]. The potencies of drugs in competing for the specific ^{125}I-BE -binding sites were determined by incubation of ^{125}I-BE 50 pmol·L^{-1} in the presence or absence of 14 concentrations of the competing drug. IC_{50} values were determined as the X intercept on a Hill plot, and K_i values were calculated by the method of Chen and Prusoff[4]. The best two-site fit for a binding curve was calculated by minimizing the sum of squares of the errors using nonlinear regression analysis. Two-site model was compared with one-site model to determine whether the increase of goodness of fit was significantly more than would be expected on the basis of chance alone[6] using a partial F test.

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

RESULTS

Scatchard analysis by ^{125}I-BE in control preparations showed K_d value of 38.5 ± 3.5 pmol·L^{-1} and B_{max} of 119.0 ± 4.5 fmol/mg protein while in the CEC-pretreated preparations ($n = 6$) showed K_d value of 58.9 ± 10.5 pmol·L^{-1}, which was not significantly different from that of the control, and B_{max} of 45.7 ± 5.2 fmol/mg protein, about 38% of the B_{max} of the control ($P < 0.01$) (Fig 1).

The competitive inhibition curve for WB4101 was best fitted for two-site model, with $35.0 \pm 4.0\%$ of high affinity sites (Fig 2A). After incubating of the preparation with CEC, the curve became best fitted for one-site model and only low affinity sites were left (Fig 2B). The competitive inhibition curve for 5-methyl-urapidil was also best fitted for the two-site model, with only $18.3 \pm 0.8\%$ of high affinity sites (Fig 2A). After pretreated with CEC, the inhibition curve was still best fitted for the two-site model, containing about $35.0 \pm 4.8\%$ of low affinity sites (Fig 2B, Tab 1).

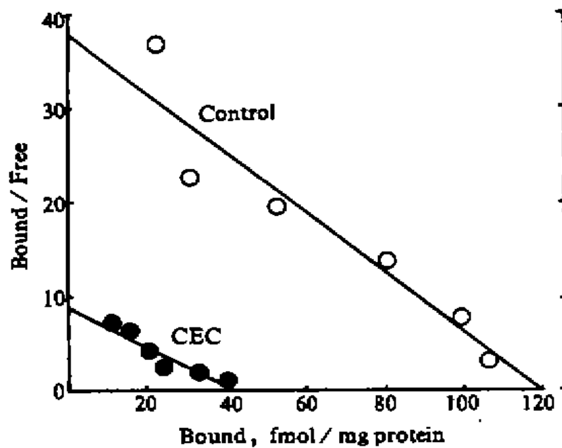

Fig 1. Effect of CEC pretreatment on ^{125}I-BE specific binding to α_1-adrenergic receptors in rat heart.

Tab 1. Two-site analysis of inhibition of specific ^{125}I-BE binding by WB4101 and 5-methyl-urapidil (5-MU) in CEC treated and control rat heart. $n = 3$, $\bar{x} \pm s$. $^bP < 0.05$ vs WB4101.

	n_H	$pK_{i\,high}$	$pK_{i\,low}$	% of $pK_{i\,high}$
Control				
5-MU	0.74	8.8±0.01	6.8±0.01	18.3±0.9^b
WB4101	0.61	9.0±0.2	7.3±0.1	35.0±4.8
CEC treated				
5-MU	0.46	9.1±0.2	6.5±0.1	65.0±12.0^b
WB4101	0.96	8.9±0.2	—	100

DISCUSSION

Two pharmacologically distinct subtypes of α_1-adrenergic receptors have been distinguished using both radioligand binding and functional assays. The α_{1A} subtype had a relative high affinity for the competitive antagonists WB4101, 5-methyl-urapidil and (+)-niguldipine and was not inactivated by alkylating agent CEC. The α_{1B} subtype has a lower affinity for these competitive antagonists and was potentially inactivated by pretreatment of CEC[1,6,7]. The cDNA for both subtypes have been cloned[8]. Recently, other two cDNA were isolated encoding α_{1C} and α_{1D} subtypes. Both α_{1C} and α_{1D} subtypes were sensitive to CEC and had relative high affinity for the com-

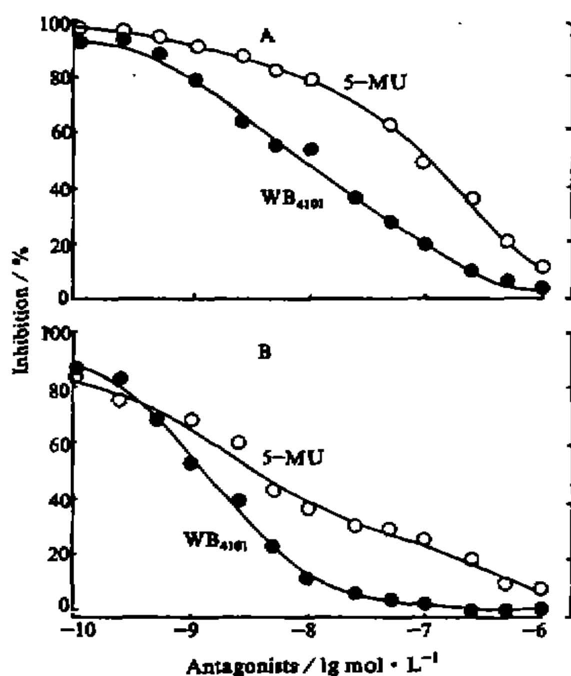

Fig 2. Inhibition of ^{125}I-BE specifice binding by 5-methyl-urapidil (○) and WB4101 (●) in control (A) and CEC-treated (B) heart preparations.

petitive antagonists, with some differences on pharmacological characteristics and tissue distribution[10]. The results of this study showed that in the membranes of rat heart, CEC pretreatment caused 62% reduction of specific binding sites to ^{125}I-BE. In the meantime, the inhibition curves for WB4101 in CEC untreated preparation showed there were 65% low affinity sites and 35% high affinity sites. The low affinity sites disappeared after the pretreatment of CEC. These results were consistent with our previous observations in rat heart[1]. However, the results did not totally fitted into the α_{1A} and α_{1B} subclassification. Because: (1) The inhibition curve for 5-methyl-urapidil also showed both high and low affinity sites. But the proportion of high affinity sites for 5-methyl-urapidil was approximately 16% lower than that for WB4101; (2) After pretreatment with CEC, WB4101 could recognize only the high affinity sites, while 5-methyl-urapidil could detecte both the high and the low affinity sites. These phenomena indicated that the high affinity sites for WB4101 may not be recognized by 5-methyl-urapidil homogeneously and there existed, in rat heart, a kind of binding sites that were insensitive to CEC, and had high affinity for WB4101 but low affinity for 5-methyl-urapidil. These pharmacological characteristics were obviously different from those of α_{1A}-, α_{1B}-, α_{1C}-, and α_{1D}-subtypes, and strongly suggested the existence of a novel subtype of α_1-adrenergic receptors in rat heart.

A strong argument for our conclusion would probably be that CEC pretreatment might not inactivate all the α_{1B} subtype receptor in the membrane preparations. But that was unlikely, because no low affinity sites for WB4101 (α_{1B}) could be distinguished after pretreatment of membrane with CEC. Furthermore, quite a lot of experiments in our laboratory have proved α_{1B} subtype can definitely be alkylated by CEC pretreatment in hypotonic buffer same as the experiment in this work[1,11].

REFERENCES

1 Han QD, Wu JH, Yang LH, Chen MZ. The two subtypes of α_1-adrenergic receptor existing in rat heart. *Chin Sci Bull* 1991; **36** : 744-7.

2 Engel G, Hoyer D. [^{125}I]BE 2254, a new high affinity radioligand for α_1-adrenoceptors. *Eur J Pharmacol* 1981; **73** : 221-4.

3 Scatchard G. The attractions of proteins for small molecules and ions. *Ann N Y Acad Sci* 1949; **51** : 660-72.

4 Cheng Y-C, Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol* 1973; **22** : 3099-108.

5 Munson PJ, Rodbard D. LIGAND: a versatile computerized approach for characterization of ligand binding systems. *Anal Biochem* 1980; **107** : 220-39.

6 Gross G, Hanft G, Rugevics C. 5-Methyl-urapidil discriminates between subtypes of the α_1-adrenoceptor. *Eur J Pharmacol* 1988; **151** : 333—5.

7 Boer R, Grassegger A, Schudt C, Glossmann H. (+)-Niguldipine binds with very high affinity to Ca^{2+} channels and to a subtype of α_1-adrenoceptors. *Eur J Pharmacol* 1989; **172** : 131—45.

8 Lomasney JW, Cotecchia S, Lorenz W, Leung W-Y, Schwinn DA, Yang-Feng TL, *et al*. Molecular cloning and expression of the cDNA for the α_{1A}-adrenergic receptor. *J Biol Chem* 1991; **266** : 6365—9.

9 Schwinn DA, Lomasney JW, Lorenz W, Szklut PJ, Fremeau Jr RT, Yang-Feng TL, *et al*. Molecular cloning and expression of the cDNA for a novel α_1-adrenergic receptor subtype. *J Biol Chem* 1990; **265** : 8183—9.

10 Perez DM, Piascik MT, Graham RM. Solution-phase library screening for the identification of rare clones: isolation of an α_{1D}-adrenergic receptor cDNA. *Mol Pharmacol* 1991; **40** : 876—83.

11 Minneman KP, Han C, Abel PW. Comparison of α_1-adrenergic receptor subtypes distinguished by chlorethylclonidine and WB4101. *Mol Pharmacol* 1988; **33** : 509—14.

482-495

一种对氯乙基可乐定不敏感对5-methyl-urapidil低亲和的新α_1肾上腺素受体亚型

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

R965.

A 摘要 用放射配体结合实验方法发现在大鼠心脏中存在一种新的α_1肾上腺素受体亚型，这种亚型不同于α_{1A}，α_{1B}，α_{1C}及α_{1D}等以往报道的α_1受体亚型，它与α_{1A}一样对WB4101具有高亲和性，但对5-methyl-urapidil呈低亲和，而且对氯乙基可乐定(CEC)不敏感.

关键词 α_1肾上腺素受体；心脏；WB4101；5-methyl-urapidil；氯乙基可乐定

BIBLID · ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Nov; 14 (6) : 495—500

Opioid, calcium, and adrenergic receptor involvement in protopine analgesia[1]

XU Qiang, JIN Rong-Luan[2], WU Yun-Yu[3]

(Department of Pharmacology of Chinese Materia Medica, [2] Department of Pharmacognosy, China Pharmaceutical University, Nanjing 210009, China)

ABSTRACT The analgesic effect of protopine (Pro), an alkaloid isolated from *Papaveraceae*, was confirmed by tail-pinch and hot-plate tests when given sc 10—40 mg·kg^{-1}, and 20—40 mg·kg^{-1} inhibited the spontaneous movements of mice. Pro 40 mg·kg^{-1} increased the sleeping rate, prolonged the sleeping duration, and shortened the sleeping latency in mice hypnotized by ip pentobarbital sodium 30 mg·kg^{-1}. Pro 10—40 mg·kg^{-1} did not affect the inflammatory reaction induced by xylene and egg white. An icv injection of Pro 20—200 μg/mouse showed a remarkable analgesic effect in mice. The icv pretreatment of naloxone 2 μg blocked the analgesic effect completely. $CaCl_2$ 40 μg/mouse (icv) or methotrexate 10 mg·kg^{-1}(ip), an agonist of Ca^{2+} channel, showed a complete blockade of the analgesia, while nifedipine 100 mg·kg^{-1}(*po*), a blocker of Ca^{2+} channel, enhanced the analgesic effect. The ip pretreatment of reserpine 4 mg·kg^{-1} reduced the Pro analgesia. Phentolamine 10 mg·kg^{-1}(ip), an α-adrenergic blocker, tended to weaken the analgesia, but propranolol 10 mg·kg^{-1}(ip), a β-blocker, did not affect it. These results suggest that Pro displays its analgesic effect mainly through the opioid and calcium systems and partly through the adrenergic mechanism.

KEY WORDS protopine; analgesia; methotrexate; nifedipine; naloxone; reserpine; phentolamine

Received 1992-07-31 Accepted 1993-05-04

[1] Project supported by the National Natural Science Foundation of China, № 38970898.

[2] Graduated in 1992.