BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Nov; 16 (6): 481—484

Differences of desensitization and hypersensitization between α_{1A}- and α_{1B}-adrenoceptors in rat isolated blood vessels[1]

DONG Er-Dan, HAN Qi-De[2] (*Institute of Vascular Medicine, The Third Hospital, Beijing Medical University, Beijing 100083, China*)

AIM: To study the differences of the agonist-induced desensitization and the reserpinization-induced hypersensitization between α_{1A}- and α_{1B}-adrenoceptors (AR) mediated vasoconstriction. **METHODS**: The thoracic aortae, mesenteric, and renal arteries of rats were isolated. The cumulative-concentration response-curve (CCRC) of vasoconstriction for NE was recorded. NE activated only α_1-AR since the perfusing Krebs solution contained propranolol 1 $\mu mol \cdot L^{-1}$ and yohimbine 0.1 $\mu mol \cdot L^{-1}$ to block β- and α_2-AR. CCRC for NE was made, preparations were pretreated with CEC 50 $\mu mol \cdot L^{-1}$ for 30 min or preincubated with NE 10 $\mu mol \cdot L^{-1}$ for 1 h then washed, and CCRC for NE was repeated. After ip reserpine 4 $mg \cdot g^{-1}$ ip, the rats were killed, the thoracic aortae and renal arteries were taken. CCRC for NE was compared with the corresponding blood vessels in control rats. **RESULTS**: Pretreatment with CEC caused reductions of the NE-induced maximal constriction by 82.5 ± 3.0 % ($P < 0.01$) and 54.2 ± 9.5 % ($P < 0.01$) in thoracic aortae and mesenteric arteries, respectively, but no effect in renal arteries. Preincubation with NE caused the α_1-AR mediated-vasoconstriction diminished 14.4 ± 5.9, 1.8 ± 0.8 and 7.3 ± 1.8 times in aortae, renal arteries, and mesenteric arteries, respectively. In reserpinized rats, the contraction in renal arteries induced by NE increased by 56 %, but showed no change in aortae. **CONCLUSION**: α_{1B}-AR mediated vasoconstriction is easier to be desensitized, while α_{1A}-AR mediated vasoconstriction is easier to be hypersensitized in rats.

KEY WORDS alpha-1 adrenergic receptors; desensitization; hypersensitivity; thoracic aorta; renal arteries; mesenteric arteries

The α_1-adrenergic receptors (AR) in rat blood vessels was subdivided into 2 subtypes, α_{1A}- and α_{1B}-AR and there were large differences on the distribution of these subtypes in various vascular smooth muscles[1]. The efficiencies of agonist-mediated vasoconstriction were also different between the 2 subtypes[2,3]. The differences on the regulation between α_{1A}- and α_{1B}-subtypes, however, have not been reported. In this study, we determined the changes of vasoconstriction induced by 2 α_1-AR subtypes under the condition of the sustained incubation of agonist or reserpinization in rats.

MATERIALS AND METHODS

Norepinephrine (NE), yohimbine, propranolol, desmethylimipramine, normetanephrine, and reserpine were from Sigma. Chloroethylclonidine (CEC) was from Research Biochemical Inc.

Wistar rats (♂, $n = 24$, 184 ± s 5 g) were killed by cervical dislocation. The thoracic aortae, mesenteric arteries, and renal arteries were cut into 3 mm rings with endothelium intact. Preparation was suspended in 10-mL bath containing Krebs solution at 37 ℃ and gassed with 95 % O_2 + 5 % CO_2. The rings

[1] Project supported by the National Natural Science Foundation of China, № 39070928 and Science Research Grant from Chinese Ministry of Public Health, № 91103015.

[2] Correspondence to Prof HAN Qi-De.

Received 1994-07-13 Accepted 1995-03-02

of aortae, mesenteric arteries and renal arteries were stretched to resting tensions of 1.0, 0.5, and 0.2 g, respectively[6,7]. To activate only the α_1-AR by NE, the Krebs solution contained yohimbine 0.1 μmol · L^{-1} to block α_2-AR, and propranolol 1 μmol · L^{-1} to block β-AR. Desmethylimipramine 0.1 μmol · L^{-1} and normetanephrine 1 μmol · L^{-1} were put in Krebs solution to block neuronal and extraneuronal uptake of NE. The cumulative-concentration-response curves (CCRC) for NE were generated. EC_{50} and 95 % confidence limits were calculated by weighted probit analysis and the $-\lg EC_{50}$ was taken as pD_2.

CEC pretreatment CCRC for NE was made, followed by 30-min washing with Krebs solution. Preparations were preincubated with CEC 50 μmol · L^{-1} for 30 min, then washed for 30 min, and CCRC for NE was repeated.

NE-preincubation To observe the vasoconstriction induced by activations of different α_1-AR subtypes, renal arteries were pretreated with CEC 50 μmol · L^{-1} for 30 min then washout for 30 min to abolish the minor α_{1B}-AR mediated response. CCRC in aortae was made in the presence of nifedipine 10 μmol · L^{-1} to block the minor α_{1A}-AR mediated effect. CCRC for NE was obtained in control, and preparations were washed for 30 min, incubated with NE 10 μmol · L^{-1} for 1 h, washout for 15 min, then the CCRC for NE was repeated.

Reserpinized pretreatment To observe the changes of vasoconstriction induced by α_1-AR in reserpinized rats, the rats were injected reserpine 4 mg · g^{-1} ip. After 48 h the rats were killed, the thoracic aortae and left renal arteries were taken. Vasoconstrictions induced by NE were observed, CCRC was compared with the corresponding blood vessels in control rats.

Statistics One way ANOVA was used to test for significance of the differences among the 3 groups. If a difference was significant, then the *t*-test or paired *t*-test was used to examine the significance between the 3 groups.

RESULTS

CEC pretreatment Pretreatment with CEC 50 μmol · L^{-1} for 30 min to irreversibly inactivated α_{1B} subtype caused reduction of the NE-induced maximal constriction by 82.5 ± 3.0 % ($P<0.01$) and 54.2 ± 9.5 % ($P<0.01$) in aortae and mesenteric arteries, respetively, but no effect in renal arteries. Sensitivities for NE were diminished in all blood vessels, with large differences in degree. EC_{50} values increased by 281-fold ($P<0.01$), 78-fold ($P<0.01$), and 6-fold ($P<0.05$) in aortae, mesenteric arteries, and renal arteries, respectively (Tab 1).

Tab 1. Effect of CEC preincubation on the NE-induced vasoconstriction in blood vessels of rats. $\bar{x} \pm s$ **(95 % confidence limits).** $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **control.**

Artery	*n*	pD_2 Control	pD_2 Treated	Maximal contraction/mg Control	Maximal contraction/mg Treated
Aorta	8	7.55±0.35 (7.52−7.58)	5.10±0.43^c (5.05−5.16)	1 180±170	210±110^c
Renal	5	6.42±0.53 (6.39−6.46)	5.60±0.19^b (5.58−5.63)	770±200	780±200^a
Mesenteric	8	7.15±0.56 (7.10−7.19)	5.25±0.41^c (5.21−5.30)	700±200	290±80^c

NE preincubation Preincubation with NE 10 μmol·L^{-1} for 1 h diminished the sensitivity for NE, the EC_{50} values being increased by 14.4 ± 5.9 times ($P<0.01$), 7.3 ± 1.8 times ($P<0.01$), and 1.8 ± 0.8 times ($P<0.05$) in aortae, mesenteric, and renal arteries, respectively. Preincubation with NE showed no significant effect on the maximal constriction induced by NE in all blood vessels (Tab 2).

Reserpine-treatment The maximal constriction induced by NE was not changed in aortae or renal arteries from the reserpinized rats. The CCRC for NE was not changed in aortae either, but the sensitivity for NE was increased in renal arteries and the EC_{50} value was reduced by 56 % ($P<0.05$) (Tab 3).

Tab 2. Effect of pre-incubation of NE 10 μmol·L^{-1} for 1 h on NE-induced vasoconstriction in rats. $\bar{x}\pm s$ (95 %: confidence limits). $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Artery	n	pD_2 Control	pD_2 Treated	Increase of EC_{50} (Fold)	Maximal contraction/mg Control	Maximal contraction/mg Treated
Aorta	6	7.48±0.16 (7.45—7.52)	6.35±0.17^c (6.32—6.38)	14.4±5.9^c	1 080±170	1 020±150^a
Renal	5	5.78±0.20 (5.76—5.81)	5.55±0.07^b (5.52—5.57)	1.8±0.8^b	920±290	950±360^a
Mesenteric	6	7.36±0.10 (7.33—7.40)	6.51±0.14^c (6.48—6.54)	7.3±1.8^c	860±150	970±120^a

Tab 3. Vasoconstrictor responses to NE in reserpinized rats. $\bar{x}\pm s$ (95 % confidence limits). $^aP>0.05$, $^bP<0.05$ *vs* control.

	n	pD_2	Maximal contraction/mg
Aorta			
Control	10	7.78±0.38 (7.75—7.81)	1 480±220
Reserpinized	10	7.57±0.43^a (7.53—7.61)	1 180±500^a
Renal artery			
Control	9	6.30±0.33 (6.27—6.34)	750±100
Reserpinized	10	6.66±0.32^b (6.62—6.70)	760±90^a

DISCUSSION

Agonists activate the receptors to produce biological effects, in the meanwhile the responses to the agonists are decreased, eg, desensitization. The previous study on adrenergic receptor desensitization was mainly focused on β, AR, for which not only the process of desensitization but also cellular mechanisms have been elucidated[5]. However, desensitizations of α_2- and α_1-AR were rarely studied.

We have previously proved that aortae contained mainly α_{1B}-receptor, renal arteries contained mainly α_{1A}-receptor and mesenteric arteries contained both receptors in rats[1]. In this experiment the effects of CEC pretreatment on vasoconstriction induced by NE again confirmed our previous suggestion. In this study we determined the NE response in the presence of nifedipine to block the minor α_{1A}-AR induced response in aortae and preincubated the renal arteries with CEC to irreversibly inactivate possibly existed the minor α_{1B}-AR[6,7]. Thus, the α_1-AR in such treated aortae and renal arteries was more representative as α_{1B}- and α_{1A}-AR, respectively.

The results showed that preincubation with NE decreased the sensitivities to NE, the order was aortae (α_{1B}-AR), mesenteric arteries (2 subtypes) and renal arteries (α_{1A}-AR). These results suggest that under the same effect of agonist the desensitization in α_{1B}-AR is more evident than α_{1A}-AR.

Reversely, after exhausting NE in sympathetic nerve terminals by reserpine, which reduced the activation of that α_1-AR in blood vessels, the sensitivity for NE increased in renal arteries (α_{1A}-AR), but did not change in aortae (α_{1B}-AR). These results indicated that α_{1A}-AR (in renal arteries) mediated vasoconstriction was easier to be hypersensitized than α_{1B}-AR (in aortae). But these results came from the experiment of isolated vasoconstriction, they reflected only the change of function on vasoconstriction. To observe the change on receptor affinity and number, the radioligand binding experiment and molecular biological technology would be further used.

In conclusion, α_{1B}-AR is more susceptible to the desensitization, while α_{1A}-AR is more susceptible to the hypersensitization. Since most blood vessels contain both α_{1A}- and α_{1B}-AR, α_{1A}-AR mediated vasoconstriction will become more prominent and function more important when ever desensitization or hypersensitization happens. This may be an important aspect of the functional significance of co-existing 2 α_1-AR subtypes in blood vessels.

REFERENCES

1 Han C, Li JL, Minneman KP. Subtypes of α_1-adrenoceptors in rat blood vessels. Eur J Pharmacol 1990; **190**: 97—104.
2 Han QD, Li JL, Bakhilov VL. Analysis of α-adrenoceptor in smooth muscle of rat caudal artery. Chin J Pharmacol Toxicol 1992; **6**: 214—7.
3 Chen YM, Han QD. Alpha adrenoceptor subtypes in smooth muscles of rat pulmonary artery. Acta Pharmacol Sin 1992; **13**: 505—8.
4 Han C, Abel PW. Neuropeptide Y potentiates contraction and inhibits relaxation of rabbit coronary arteries. J Cardiovasc Pharmacol 1987; **9**: 675—81.
5 Hausdorff WP, Caron MG, Lefkowitz RJ. Turning off the signal: desensitization of β-adrenergic receptor function. FASEB J 1990; **4**: 2881—9.
6 Minneman KP, Han C, Abel PW. Comparison of α_1-adrenergic receptors revealed by chlorethylclonidine. Mol Pharmacol 1988; **33**: 509—14.
7 Han C, Abel PW, Minneman KP. α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature 1987; **329**: 333—5.

大鼠血管 α_1肾上腺素受体两种亚型减敏与增敏过程的差别

董尔丹，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）

目的：研究 α_1肾上腺素受体(α_1-AR)不同亚型之间激动剂诱导的减敏和利血平化诱导的增敏过程的差别. **方法**：采用大鼠离体血管收缩功能实验，观察主动脉、肾动脉和肠系膜动脉在 CEC 100 μmol·L^{-1}温育30 min 后，NE 介导的累积浓度收缩曲线(CCRC)的变化，以观察不同血管的亚型差异；在观察减敏时，血管用 NE 10 μmol·L^{-1}预温育1 h，洗出后做 NE 的 CCRC；在观察增敏的差异时，大鼠用 reserpine 4 mg·kg^{-1} ip 48 h 后，观察 NE 诱导的 CCRC 的变化. 本实验在灌流液中含 yohimbine 和 propranolol 以阻断 α_2-和 β-AR，这样 NE 仅激活 α_1-AR. **结果**：CEC 100 μmol·L^{-1}温育30 min，使 NE 介导的主动脉，肠系膜动脉的最大收缩分别降低82.5±3.0 %和54.2±9.5 %，而对肾动脉则无影响，再次肯定主动脉主要含 α_{1B}-AR、肾动脉主要含 α_{1A}-AR，肠系膜动脉兼含两种亚型. NE 10 μmol·L^{-1}温育1 h 后，NE 介导的主动脉，肾动脉和肠系膜动脉收缩的敏感性降低，EC_{50}分别增加了14.4±5.9，1.8±0.8和7.3±1.8倍；而在利血平化大鼠 NE 介导肾动脉收缩反应增加了56 %，主动脉则无变化. **结论**：α_{1B}-AR 亚型介导的反应易发生减敏；α_{1A}-AR 亚型介导的反应易发生增敏.

关键词 α_1肾上腺素受体；脱敏；增敏；胸主动脉；肾动脉；肠系膜动脉