Influence of stretch on α_1 receptor agonist phenylephrine regulated vasoconstriction in rabbit regional arteries

REN Lei-Ming*, ZHANG Miao, YUAN Zhi-Fang, ZHU Zhong-Ning, SHI Chen-Xia
(*Department of Pharmacology, School of Pharmacy, Hebei Medical University, Shijiazhuang* 050017, *China*)

Abstract: **AIM** To investigate how to determine the optimal preloads in the study of α_1 receptor agonist phenylephrine (Phe) regulated vasoconstriction in the rabbit isolated arteries. **METHODS** Vasoconstrictive responses to Phe were recorded in the rabbit renal, femoral, saphenous, mesenteric, splenic and ear arteries. **RESULTS** The resting tension in various arterial rings was increased with increasing preload from 1.0 g to 5.0 g in a linear manner. The vasoconstrictive responses to KCl (120 $mmol \cdot L^{-1}$) in the regional arteries were increased with increasing preload, however, the final plateau of the E_{max} of KCl was not observed in these regional arteries, with the exception of the ear artery. In the experiment with Phe (0.01 – 100 $\mu mol \cdot L^{-1}$) as a vasoconstrictive agent, the EC_{50} values of Phe ($EC_{50 \cdot Phe}$) in the rabbit mesenteric, splenic and saphenous arteries were changed largely by changing preload, however there was an optimal point where the $EC_{50 \cdot Phe}$ value and its standard deviation were minimal among the five values corresponding to 1.0 g to 5.0 g preloads in each artery. **CONCLUSION** The EC_{50} values of Phe in the rabbit isolated arteries are influenced by preloads obviously. In the determination of optimal preload in isolated arterial preparations, particularly in the study of receptor regulated function, the minimal EC_{50} value and its standard deviation of corresponding-receptor agonist should be considered as a crucial factor.

Key words: adrenergic alpha-agonists; phenylephrine; arteries; stretch; vasoconstriction; rabbits

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Biography: REN Lei-Ming(1956 –), male, native of Hebei Province, Professor, main research field is cardiovascular pharmacology.
* Corresponding author. Tel: (0311)6044121 ext 5170, Fax: (0311)6048177, E-mail: ren-leiming@263.net

Isolated arterial ring preparations are often used in physiological and pharmacological studies. However, in many experiments with blood vessels obtained from even the same region of the same species, the optimal preloads (stretch levels) and the methods to determine the optimal preloads were different[1-3]. In addition, the reported parameters of concentration-dependent response curve even for the same α-adrenoceptor agonist (norepinephrine, NE) in the same tissue (rabbit isolated ear artery) from individual research lab were significantly different from each other[1-3]. In the present study, we observed the cumulative concentration-dependent response curves for phenylephrine (Phe) in six kinds of the isolated regional arteries of the rabbit, and analyzed the influence of 1 – 5 g stretch levels (preloads) on the concentration of 50% maximum effect (EC_{50}) values of Phe ($EC_{50 \cdot Phe}$), which represented the affinity of Phe to functional α_1-adrenoceptor in these arteries.

1 MATERIALS AND METHODS

1.1 Rabbits

Male New-Zealand white rabbits (2.5 – 3.5 kg) were obtained from the Experimental Animal Center of Hebei Medical University (Certificate No 0059).

1.2 Chemical

Phe, obtained from Sigma Chemical Co., was dissolved in distilled water.

1.3 Arterial preparation

Rabbits were stunned by a blow, then exsanguinated. The renal, femoral, saphenous, mesenteric, splenic and ear arteries were excised and cleaned of excess connective tissue and fat. Ring

segments (4 mm in length) with endothelium were mounted horizontally in a 10 mL organ bath by carefully inserting a tungsten wire through the lumen of the vessel ring and anchoring it to a stationary support. Another wire similarly inserted, was connected to an isometric tension transducer, and responses were recorded on a polygraph (ERT-884, Youlin Electron Co., Kaifeng).

Preloads of 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0 g were applied to the renal, femoral, saphenous, mesenteric, splenic and ear arterial rings, respectively. In order to avoid the influence of individual difference of the rabbits, the four arterial rings obtained from the same region of the same rabbit were given four different preloads respectively, and only one preload was used in one preparation. Preparations under the used-preload were allowed to equilibrate for 1 h in physiological solution of the following composition (mmol·L^{-1})[4]: NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52, pH 7.2. The solution was maintained at 37℃ and aerated with 95% O_2 and 5% CO_2.

1.4 Drug administration

Phe (0.01 – 100 μmol·L^{-1}) was added cumulatively to the organ bath to construct concentration-response curves of the vasoconstriction. The concentration-response curves for Phe were repeated three times in each preparation at 35 min interval, and the first set of data was not used in analysis. A single concentration (120 mmol·L^{-1}) of KCl was added to the preparation in the end of each experiment.

1.5 Statistics

Data were expressed as $\bar{x} \pm s$. The EC_{50} values were calculated with the equation: $\log[E/(E_{max} - E)] = \log c - \log K$ (E, response; E_{max}, maximal response; c, Phe concentration; K, equilibrium dissociation constant)[5]. Duncan′s multiple range test (with computer program of PHARM/PCS-Version 4) was used to evaluate any differences among more than two groups. Two way ANOVA was used to evaluate a difference between two concentration-dependent curves for Phe.

2 RESULTS

2.1 Effects of different preloads on the resting tension of the six regional arteries

The resting tensions of the renal, femoral, saphenous, splenic, mesenteric, and ear arterial rings were increased with the increase in preloads (1.0 to 5.0 g) from 0.5 – 0.86 g to 3.66 – 3.92 g ($P < 0.05$, Duncan′s multiple range test), and the increase in resting tensions in these arteries under the different preloads was in a linear manner ($r = 0.9891 - 0.9990$, $P < 0.01$, Fig 1).

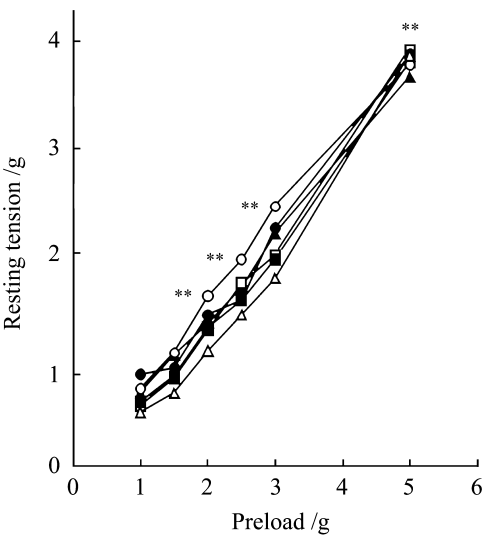

Fig 1. Resting tension in rabbit renal (△), femoral (□), saphenous (●), splenic (▲), ear (○) and mesenteric (■) arteries with different preloads. Points represent the mean values, $n = 5$. ** $P < 0.01$, compared with preload 1.0 g for each artery.

2.2 Effects of different preloads on the maximal contractile responses to Phe in the six regional arteries

Under each preload, the second and the third concentration-response curves for Phe were not significantly different from each other ($P > 0.05$, two way ANOVA, data not shown) in the six regional arteries. With the increase in preloads (1.0 to 5.0 g), the maximal vasoconstrictive responses to Phe ($E_{max \cdot Phe}$, calculated from the second concentration-response curve) in these arteries were increased significantly ($P < 0.05$, Duncan′s

multiple range test) in different manners. In the ear artery $E_{max \cdot Phe}$ value increased in a monophase manner, and it reached plateau at 3 g preload (Fig 2). In other five arterial rings, the $E_{max \cdot Phe}$ values were increased in a biphasic manner, and the $E_{max \cdot Phe}$ values reached the first plateau at 1.5 g in the splenic artery, at 2 g in the mesenteric artery, at 2.5 g in the saphenous and femoral arteries, and at 3 g in the renal artery (Fig 2).

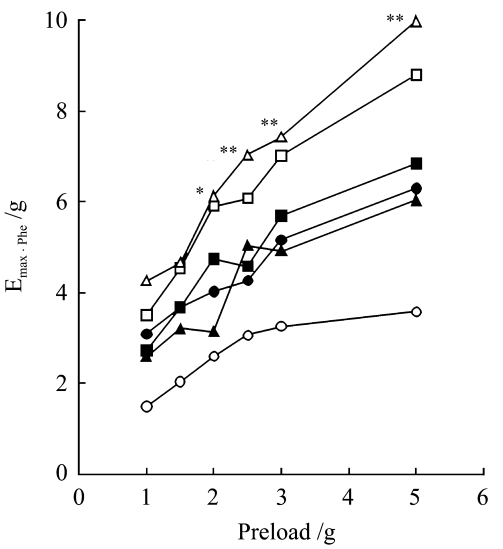

Fig 2. Maximal contractile response to phenylephrine ($E_{max \cdot Phe}$) in rabbit renal (△), femoral (□), saphenous (●), splenic (▲), ear (○) and mesenteric (■) arteries with different preloads. Phe (0.01 – 100 μmol·L^{-1}) was added cumulatively to the organ bath to construct concentration-response curves of the vasoconstriction. Points represent the mean values, $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with preload 1.0 g for each artery.

2.3 Effects of different preloads on the $EC_{50 \cdot Phe}$ in the six regional arteries

The $EC_{50 \cdot Phe}$ to produce concentration-dependent contractile responses did not change significantly ($P > 0.05$, Duncan's multiple range test) in each one of the six regional arteries under different preloads (1 to 5 g), because in each artery there was at least one or two of the standard deviation values which were much bigger (Tab 1). On the other hand, in each one of the six regional arteries under different preloads (1 to 5 g), there was an optimal point where the $EC_{50 \cdot Phe}$ value and its standard deviation were the least among the six values with the exception of mesenteric artery (Tab 1). In the mesenteric artery, the $EC_{50 \cdot Phe}$ values could be calculated only at 1.5 and 2.0 g preloads with the equation (Tab 1), because the vasoconstrictive responses to Phe were not able to reach the maximal response under the preloads of 1.0 g and 2.5 – 5.0 g.

2.4 Effects of different preloads on the maximal contractile responses to KCl ($E_{max \cdot KCl}$) in the six regional arteries

With the increase in preloads (1.0 to 5.0 g), the $E_{max \cdot KCl}$ to 120 mmol·L^{-1} KCl in the six regional arteries was increased significantly ($P < 0.05$, Duncan's multiple range test). In the ear artery, $E_{max \cdot KCl}$ value was increased to a plateau at 2.5 g of preload. However, in other five arterial rings, the $E_{max \cdot KCl}$ values were increased in a biphasic manner. In the splenic artery, $E_{max \cdot KCl}$ value reached to the first plateau at 2.5 g, and it reached the first plateau at 2.0 g in the mesenteric, saphenous, femoral and renal arteries (Tab 2).

3 DISCUSSION

Vessel ring preparations are commonly used in the study of physiology and pharmacology. However, the optimal stretch levels in resting condition (optimal preloads) were quite different even in the study using the same preparation and agent[6,7]. Zhang, *et al*[6] reported a same preload (0.5 g) applied to the rabbit renal, mesenteric, pulmonary and femoral arteries without considering the heterogeneity of the regional arteries[8].

Generally, optimal preloads are usually determined with two kinds of methods. Firstly, vessel rings under different preloads were exposed to maximal concentration of KCl, and the maximal contractile response to the minimal preload was chosen[8]. Secondly, vessel rings under different preloads were exposed to single concentration to be near EC_{50} of vasoactive agent (NE or Phe), and the preload producing large and reproductive

Tab 1. EC_{50} values of phenylephrine ($EC_{50\cdot Phe}$) in the rabbit renal, femoral, saphenous, splenic, ear and mesenteric arteries with different preloads

Preload /g	$EC_{50\cdot Phe}/\mu mol\cdot L^{-1}$					
	Renal	Femoral	Saphenous	Mesenteric	Splenic	Ear
1.0	3.7 ± 1.2	1.9 ± 1.1	13 ± 16	–	13 ± 10	1.3 ± 1.2
1.5	3.1 ± 1.4	1.4 ± 0.6	12 ± 13	30 ± 21	17 ± 14	1.9 ± 1.2
2.0	3.4 ± 1.9	1.1 ± 0.4	6.8 ± 7.0	52 ± 37	7.5 ± 3.1	0.9 ± 0.4
2.5	4.6 ± 2.4	0.8 ± 0.4	2.8 ± 1.5	–	32 ± 32	2.5 ± 3.2
3.0	2.9 ± 1.0	0.7 ± 0.3	5.3 ± 5.3	–	28 ± 39	2.3 ± 1.7
5.0	2.6 ± 0.8	0.8 ± 0.3	5.4 ± 5.6	–	33 ± 64	1.1 ± 0.4

Phe (0.01 – 100 $\mu mol\cdot L^{-1}$) was added cumulatively to the organ bath to construct concentration-response curves of the vasoconstriction. (–): The values of EC_{50} could not be calculated with the equation. $\bar{x} \pm s$, $n = 5$.

Tab 2. E_{max} to KCl (120 mmol·L^{-1}) in the rabbit renal, femoral, saphenous, splenic, ear and mesenteric arteries with different preloads

Preload /g	$E_{max\cdot KCl}$/g					
	Renal	Femoral	Saphenous	Mesenteric	Splenic	Ear
1.0	3.1 ± 0.4	2.4 ± 0.7	1.92 ± 0.29	1.4 ± 0.5	1.00 ± 0.26	0.96 ± 0.21
1.5	3.6 ± 0.4	2.9 ± 0.7	2.2 ± 0.3	2.2 ± 0.5	1.04 ± 0.25	1.34 ± 0.15
2.0	4.0 ± 1.1	4.2 ± 0.5**	2.6 ± 0.3	2.5 ± 0.4*	1.0 ± 0.4	1.7 ± 0.3*
2.5	3.8 ± 1.2	4.1 ± 0.3**	2.6 ± 0.6	2.52 ± 0.28*	1.8 ± 0.7	1.80 ± 0.20**
3.0	4.3 ± 1.0	4.8 ± 0.8**	3.4 ± 0.3**	3.3 ± 0.7**	1.3 ± 0.6	1.76 ± 0.09*
5.0	4.6 ± 1.2*	4.7 ± 1.0**	3.8 ± 1.1**	3.5 ± 0.9**	2.7 ± 1.2**	1.8 ± 0.8**

$\bar{x} \pm s$, $n = 5$. * $P < 0.05$, ** $P < 0.01$, compared with the preload 1.0 g.

contractile response was chosen[9-12].

In the present experiment with $E_{max\cdot KCl}$ value as a parameter, although the $E_{max\cdot KCl}$ value in the ear artery increased to a plateau at 2.5 g of preload, $E_{max\cdot KCl}$ values in other five arterial rings reached to the first plateau at 2.0 – 2.5 g. With further increase in the preload to 5.0 g in the mesenteric, splenic, saphenous, femoral and renal arteries, $E_{max\cdot KCl}$ values were increased significantly, and the second plateau was not observed. Therefore, in the present study, it was difficult to determine optimal preload using maximal concentration of KCl in the rabbit mesenteric, splenic, saphenous, femoral and renal arteries, with the exception of the ear artery.

In the present experiment with Phe as a vasoconstrictive agent, we found one point where the $EC_{50\cdot Phe}$ value and its standard deviation was the minimal one among the five values corresponding to 1 – 5 g preloads in each of the rabbit mesenteric, splenic, saphenous, femoral and ear arteries. Theoretically, the $EC_{50\cdot Phe}$ value reflects its affinity to α_1-adrenoceptor. However, the $EC_{50\cdot Phe}$ values in the rabbit mesenteric, splenic and saphenous arteries were changed within 10 – 25 $\mu mol\cdot L^{-1}$ by changing preload, indicating that the applied preload might affect the binding of Phe to α_1-adrenoceptor. On the basis of the present results, we considered that optimal preload for the binding of Phe to α_1-adrenoceptor should be the preload at which the $EC_{50\cdot Phe}$ value and its standard deviation were the minimal.

In the rabbit ear artery, Saville, *et al*[2] reported that EC_{50} of NE ($EC_{50\cdot NE}$) was 0.938

$\mu mol \cdot L^{-1}$, and Ziganshin, *et al*[3] reported that E_{max} and pD_2 value of NE were (3.68 ± 0.35) g and 6.07 ± 0.13. In order to test our method to determine optimal preload, the rabbit ear artery with preload 2.0 g was used to construct the concentration-dependent response curve for NE (n = 6), and its E_{max}, $EC_{50 \cdot NE}$ and pD_2 value were (3.59 ± 0.48) g, (0.93 ± 0.19) $\mu mol \cdot L^{-1}$ and 6.04 ± 0.09, which were consistent with the above publications[2, 3]. On the other hand, Xu, *et al*[13] reported a significantly different result in the rabbit ear artery with 1.5 g preload. Even in the same lab, Burnstock, *et al*[14] reported that in rabbit saphenous artery with resting tension of 0.75 g, E_{max} and pD_2 value of NE were (3.32 ± 0.23) g and 5.05 ± 0.04, whereas Ziyal, *et al*[15] reported an obviously different results in which E_{max} and pD_2 value of NE were (4.40 ± 0.33) g and 6.13 ± 0.08 in the same preparation with 1.0 g preload.

Hence, in determination of optimal preload in isolated arterial preparations, particularly in the study of functional receptor, the minimal EC_{50} value and its standard deviation of EC_{50} value of corresponding-receptor agonist should be considered as a crucial factor.

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牵拉对 α_1 受体激动剂苯肾上腺素诱发兔离体血管收缩反应的影响

任雷鸣，张 淼，袁志芳，朱忠宁，师晨霞
（河北医科大学药学院药理学研究室，石家庄 050017）

摘要：目的 研究在 α_1 受体激动剂苯肾上腺素（Phe）诱发血管收缩反应实验中，如何确定动脉标本的最适前负荷。**方法** 采用兔离体肾动脉、股动脉、隐动脉、肠系膜动脉、脾动脉和耳中央动脉环标本的等长张力纪录法。**结果** 在 1.0～5.0 g 前负荷条件下，随前负荷增加，各血管环静息张力呈线性增加。KCl（120 mmol·L^{-1}）引起各动脉的收缩随前负荷的增加而增强，但除耳动脉外，在其他动脉标本上未观察到 KCl 最大收缩反应的最终坪值点。以 Phe（0.01～100 μmol·L^{-1}）为收缩剂时，在肠系膜动脉，脾动脉和隐动脉，Phe 的 EC_{50}（$EC_{50\cdot Phe}$）值随前负荷增加（1.0～5.0 g）而明显改变；但是，各组标本在不同前负荷下的 $EC_{50\cdot Phe}$ 值中存在一个最小值，其标准差亦很小。**结论** 前负荷明显影响血管的 $EC_{50\cdot Phe}$ 值测定。确定离体血管标本最适前负荷，特别是研究受体反应时，应以受体激动剂的 EC_{50} 值及其变异程度最小作为关键指标。

关键词： 肾上腺素 α 受体激动剂；苯肾上腺素；动脉；牵拉；血管收缩；兔

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