ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5): 445 - 449
http://www.chinaphar.com

Contractile responses of diabetic rat aorta to phenylephrine at different stages of diabetic duration[1]

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KEY WORDS *experimental diabetes mellitus; thoracic aorta; phenylephrine; streptozocin; cyclopiazonic acid; calcium*

ABSTRACT

AIM: *To investigate the time-dependent changes in contractile responses of aorta to phenylephrine (Phe) in diabetic rats and age-matched control, and its possible mechanism.* **METHODS**: At stages of 2-, 6-, and 12-week diabetic duration, aortic rings were studied for contractile responses to agonists *in vitro*. **RESULTS**: At the stage of 2-week diabetic duration, contractile responses to lower concentrations of phenylephrine were increased ($P < 0.05$), but the maximal contraction of phenylephrine did not change. At the stage of 6-week diabetic duration, contractile responses to phenylephrine were increased ($P < 0.01$) at each concentration, and the maximal contraction was increased by approximately 40 %. However, at the stage of 12-week diabetic duration: 1) the maximal contractile response to Phe 10 $\mu mol \cdot L^{-1}$ was decreased ($P < 0.05$), 2) in Ca^{2+} free edetic acid medium, Phe 10 $\mu mol \cdot L^{-1}$-induced transient contraction was also decreased ($P < 0.05$), 3) in Ca^{2+} free edetic acid medium, in the presence of nifedipine 10 $\mu mol \cdot L^{-1}$ and Phe 10 $\mu mol \cdot L^{-1}$, the Ca^{2+} repletion-caused contraction was not different from control, 4) in normal medium, cyclopiazonic acid (CPA) 10 $\mu mol \cdot L^{-1}$-induced contraction was decreased ($P < 0.01$). **CONCLUSION**: The results suggested that contractile responses to phenylephrine in diabetic rat aorta changed with the development of diabetes, and the changes of functional Ca^{2+} store sizes and Ca^{2+} entry mainly through voltage-dependent Ca^{2+} channels were responsible for the alterations of contractile responses to phenylephrine in diabetes.

INTRODUCTION

Contractile responses of aortic rings to most of agonists are known to change in diabetes[1]. This kind of changes reflects the early dysfunction of blood vessels and is related with the diabetic complications. Although it is widely known that contractile response of aortic rings to the physiologic stimulator, phenylephrine (Phe), is enhanced[2-4], some reports show no change and even decreased contractions[5,6]. The alterations in contractile responses may be related to the differences in the duration and degree of metabolic abnormalities in diabetes at the time of period[1].

Both extracellular and intracellular Ca^{2+} contribute to the Phe-induced contraction. Studies have suggested that Ca^{2+} handling systems are altered in diabetes[7], but the mechanism is still far from being clear.

In this study, we investigated the time-dependent changes in contractile responses to Phe in diabetic and its possible mechanism.

MATERIALS AND METHODS

General procedures In Wister ♂ rats obtained from the Animal Center of Sun Yat-Sen University of Medical Sciences (Grade Ⅱ, Certificate № 26-98A011) weighing 200 - 250 g, streptozocin (STZ 65 mg/kg) dissolved in citrate buffer 0.1 $mol \cdot L^{-1}$ (pH = 4.5) was injected (ip) to induce diabetes. Controls were injected with a vehicle. A drop of tail blood was obtained at 1 week after administration of STZ and at the time of period to verify hyperglycaemia using a Boehringer Mannheim glucometer [(5.0 ± 1.1) $mmol \cdot L^{-1}$ in control, $n = 50$, (28 ± 13) $mmol \cdot L^{-1}$ in diabetes, $n = 50$].

After two, six, and twelve weeks, the rats were de-

[1]Project supported by the Science Foundation of Guangdong Province (99M01304G), and the National Natural Science Foundation of China (№ *39970849*).
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Received 2000-06-09 *Accepted* 2001-02-02

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
E-mail aps@mail.shonc.ac.cn Phn/Fax 86-21-6474-2629

capitated, and the descending thoracic aortae were carefully isolated, removed into 37 ℃ modified Krebs' solution containing (in mmol·L^{-1}) NaCl 137, KCl 5.4, $CaCl_2$ 2.0, $MgCl_2$ 1.1, NaH_2PO_4 0.4, glucose 5.6, and $NaHCO_3$ 11.9 (pH = 7.2). The solution was aerated with 95 % O_2 and 5 % CO_2. The aortic segments were carefully cleaned of fat and loose connective tissue, and sectioned into 3-mm rings. At the most two rings were obtained from each aorta. The endothelium was removed by gently rubbing on the fingers, and this was confirmed in pilot study by the lack of relaxation of preparations precontracted with Phe 0.3 μmol·L^{-1} upon acetylcholine 10 μmol·L^{-1}.

Isolated aortic rings were suspended between parallel hooks in 4-mL baths which were thermoregulated at 37 ℃ modified Krebs' solution. Resting tension was set at a level of 1.5 g. A recorder via force-displacement transducers recorded changes in isometric tension. At the end of the experiment, the rings were blotted dry and weighed. Contractile forces were calculated as g·g^{-1} wet tissue.

Individual protocols Each ring was equilibrated for 2 h with the resting tension of 1.5 g. During the equilibration, the rings were washed every 20 min. Before taking data, the rings were stimulated with KCl 100 mmol·L^{-1}, 60 mmol·L^{-1}, and 60 mmol·L^{-1} to make sure that the latter KCl 60 mmol·L^{-1} responses were reproducible. Subsequently, rings were washed, and the tension was allowed to return to baseline for 45 min. Then, contractile responses to Phe were studied. For the Ca^{2+}-free test, Ca^{2+} was omitted in the solution. The rings were washed six times with Ca^{2+}-free solution, and egtazic acid 50 μmol·L^{-1} was added to the baths for 10 min. This level of egtazic acid was sufficient to chelate the contaminating Ca^{2+} in the nominally Ca^{2+}-free medium without any significant membrane-destabilizing effect for 30 min[8].

Drugs Phenylephrine, CPA, egtazic acid, and nifedipine were purchased from Sigma (St Louis, MO, USA).

Statistics All values were expressed as $\bar{x} \pm s$. Data analysis was performed using worksheet program, Microsoft Excel t test (unpaired), $P < 0.05$ was considered statistically significant.

RESULTS

At the 6-week period, diabetic rats lost approximately 15 % body weight (from 225 g ± 25 g to 200 g ± 21 g), however, control rats gained approximately 30 % body weight (up to 300 g ± 18 g). At the 12-week period, control rats gained approximately 90 % body weight (up to 420 g ± 14 g), there was no significant changes in body weight of 12-week diabetic rats as compared with that of 6-week diabetic rats.

At the 2-week period, cumulative concentration responses to Phe were increased ($P < 0.01$) at low concentrations (0.01, 0.03 μmol·L^{-1}). The EC_{50} of Phe was decreased about 4.8 folds in diabetic aortae as compared with control [(0.04 ± 0.01) μmol·L^{-1} in diabetic aorta *vs* (0.19 ± 0.06) μmol·L^{-1} in control] (Fig 1).

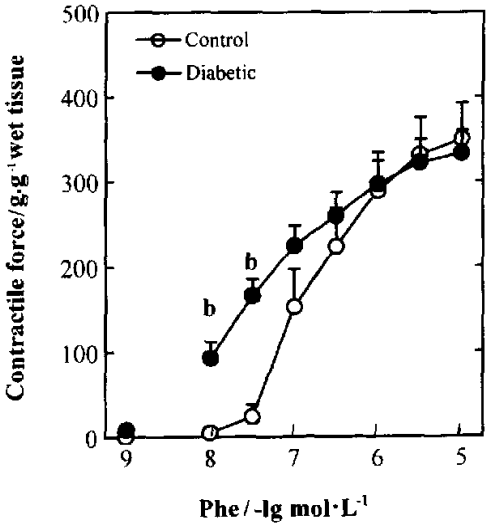

Fig 1. Cumulative concentration response curves for phenylephrine in aorta of 2-week diabetic rat and age-matched control. Data represents 4 rings of 4 rats of both control and diabetic rats. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control.

With the development of diabetes, the contractile responses to Phe were increased at each concentration of Phe at 6-week period, and the maximum contraction was increased by approximately 40 % (in g·g^{-1} wet tissue, 183 ± 26 in control, $n = 12$ *vs* 260 ± 27 in diabetic rats, $n = 15$, $P < 0.01$) (Fig 2).

Since the contractile responses to Phe were increased in 6-week diabetes, we used the maximal concentration of Phe (10 μmol·L^{-1}) to see whether this kind of enhancement increased further in 12-week period. However, the result showed that it was decreased (in g·g^{-1} wet tissue, 236 ± 32 in diabetic, $n = 15$ *vs* 287 ± 33 in control, $n =$

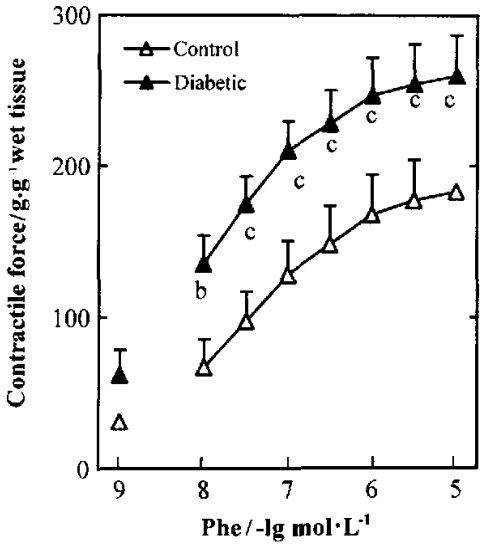

Fig 2. Cumulative concentration response curves for phenylephrine in aortas of 6-week diabetic rat and age-matched control. Control: $n = 12$; Diabetic: $n = 15$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

20, $P < 0.05$) (Tab 1).

Consistent with the decrease in contractile response to Phe 10 μmol·L^{-1} in diabetic aortae at the 12-week period, the contractile response to CPA 10 μmol·L^{-1} was also decreased in diabetic aortae (in g·g^{-1} wet tissue, 85 ± 27 in diabetic, $n = 15$ *vs* 163 ± 36 in control, $n = 14$, $P < 0.01$) (Tab 1). CPA 10 μmol·L^{-1} usually triggered an oscillatory contraction in diabetic, but not in control aorta (data not shown). In Ca^{2+} free egtazic acid (50 μmol·L^{-1}) medium, Phe 10 μmol·L^{-1}-caused contraction, which was presumably due to the release of intracellular Ca^{2+}, was also decreased (in g·g^{-1} wet tissue, 45 ± 19 in diabetic aorta, $n = 18$ *vs* 65 ± 26 in control, $n = 13$, $P < 0.05$) (Tab 1).

In the Ca^{2+} free egtazic acid containing medium with nifedipine, Ca^{2+} repletion-caused contraction in 12-week diabetic aorta, presumably due to Ca^{2+} influx by non-voltage-dependent Ca^{2+} channels, was not different from control (in g·g^{-1} wet tissue, 61 ± 24 in control, $n = 7$ *vs* 64 ± 26 in diabetic, $n = 10$, $P > 0.05$) (Tab 1). At the 6-week period, in the presence of nifedipine, the cumulative concentration response curve of Phe in diabetic aorta was also not different from control in normal Krebs' solution (data not shown).

DISCUSSION

In this study, we found that the contractile responses to Phe in diabetic rat aorta were changed at different time of diabetic durations. In the 2-week period, the increased sensitivity of α_1-adrenergic receptor of aortic rings to Phe may account for the decreased EC_{50} and increased contractile responses to low concentrations of Phe in the diabetic aorta. In 6-week period, contractile responses to Phe were increased at each concentration of Phe, but in the presence of nifedipine 1 μmol·L^{-1}, there was no difference observed between two groups (data not shown). In 12-week period, in the presence of 1 μmol·L^{-1} nifedipine, contractile response to Ca^{2+} repletion in Ca^{2+}-free egtazic acid medium was not altered in diabetic aorta. This supported the postulation that the Ca^{2+} influx mainly through voltage-dependent Ca^{2+} channels was responsible for the increased contractile responses to Phe[2,3].

Up to now the mechanisms related to Ca^{2+} influx in smooth muscle are not quite clear. The contractions caused by agonists like Phe are considered to be the result of Ca^{2+} release from intracellular Ca^{2+} stores and the following Ca^{2+} influx. The capacitative Ca^{2+} influx (store-operated Ca^{2+} influx), known to occur following agonist activation and the depletion of Ca^{2+} stores, contains voltage-dependent and voltage-independent components[9]. In rat aorta the majority of the contractions to Phe and CPA are nifedipine sensitive. Although store-operated Ca^{2+} entry is believed to be important only for the re-filling of the depleted sarcoplasmic reticulum (SR) and not as a source of activator of Ca^{2+} for the contractile mechanisms, there are many observations that the Ca^{2+}-ATPase inhibitors like CPA not only increase Ca^{2+} influx but also increase the tone in a variety of smooth muscle preparations[10].

In the 12-week period, the maximal contraction by Phe 10 μmol·L^{-1} in normal medium was decreased. Similarly, the maximal transient contraction by Phe 10 μmol·L^{-1} in Ca^{2+} free egtazic acid medium was also decreased. This transient contraction was presumably due to Ca^{2+} release from intracellular Ca^{2+} stores. Furthermore, CPA 10 μmol·L^{-1}-induced contraction in normal medium was decreased substantially. CPA, as an inhibitor of SR Ca^{2+} pump, causes the depletion of SR Ca^{2+} stores and the following capacitative Ca^{2+} entry.

In the 6-week period, consistent with the increased contractile responses to Phe, contractile response to CPA was also increased in diabetic aorta (control *vs* diabetic,

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Tab 1. Agonists induced maximal contractions of rat aortae in 12-week diabetic and age-matched control. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Agonists and conditions	Maximal contractions/g·g^{-1} wet tissue	
	Control (n)	Diabetic (n)
Phe 10 μmol·L^{-1} normal medium	287 ± 34 (15)	236 ± 32 (20)^b
CPA 10 μmol·L^{-1} normal medium	163 ± 36 (14)	85 ± 27 (15)^c
Phe 10 μmol·L^{-1} in Ca^{2+} free egtazic acid medium	65 ± 26 (13)	45 ± 19 (18)^b
Phe 10 μmol·L^{-1} + nifedipine 1 μmol·L^{-1} in Ca^{2+} free egtazic acid medium	61 ± 24 (7)	64 ± 26 (10)

213 ± 41 *vs* 284 ± 55, $n = 3$). There is a report suggesting that the increased release of intracellular Ca^{2+} appeared to contribute to enhanced responses to α_1-adrenoceptor stimulation in diabetes. Therefore, we can suggest that the functional Ca^{2+} stores and the Ca^{2+} influx through store-operated Ca^{2+} channels may be impaired in diabetes, and this contributed to the altered contractile response to Phe.

In conclusion, this study has suggested that: (1) contractile responses of aortic rings to Phe in diabetic rats changed with the development of diabetes. (2) In 12-week of diabetes, the functional Ca^{2+} store sizes diminished, and the capacitative Ca^{2+} influx mainly through voltage-dependent Ca^{2+} channels decreased. Diminished functional Ca^{2+} store sizes and capacitative Ca^{2+} influx were most likely related with the decreased contractile responses to Phe. (3) The functional Ca^{2+} store sizes and capacitative Ca^{2+} influx seemed to be the main regulators of smooth muscle tone, and had an important relationship with the altered contractile response to Phe in diabetes.

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糖尿病大鼠病程的不同时期主动脉平滑肌对苯肾上腺素的收缩反应[1]

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关键词　实验性糖尿病；胸主动脉；苯肾上腺素；链尿菌素；环匹阿尼酸；钙

目的：研究随着糖尿病的发生发展，大鼠主动脉平滑肌对苯肾上腺素等激动剂收缩反应的变化及其可能机制．**方法**：用链尿菌素诱导糖尿病后，在第 2、6、12 周，取主动脉环进行实验观察．**结果**：苯肾上腺素的浓度依赖性收缩反应曲线，与对照相比：在第 2 周，低浓度时(0.01 – 0.03 μmol·L^{-1})明显增加($P < 0.01$)，最大反应无明显变化；在第 6 周，各浓度点均显著增加，且最大收缩反应增加约 40 %；然而，在第 12 周 1)苯肾上腺素 10 μmol·L^{-1}引起的最大收缩反应趋向降低($P < 0.05$)，2) 在无 Ca^{2+}液，也较对照明显减小($P < 0.05$)，3) 在无 Ca^{2+}液，在尼非地平 1 μmol·L^{-1}和苯肾上腺素 10 μmol·L^{-1}存

在下，复 Ca^{2+} 引起的收缩在两组间的差异未见显著性，4）在正常 Krebs' 液，环匹阿尼酸 10 $\mu mol \cdot L^{-1}$ 引起的收缩反应较对照也显著减小（$P < 0.001$）. **结论**：（1）在糖尿病的第 2 周，平滑肌 α_1-肾上腺素能受体的敏感性增加. （2）糖尿病大鼠主动脉平滑肌对苯肾上腺素收缩反应的异常变化，与通过电压依赖性钙通道的 Ca^{2+} 内流大小、胞内功能性 Ca^{2+} 池大小及其胞内 Ca^{2+} 池耗竭后所引起的充电性内流变化密切相关.

（责任编辑　朱倩蓉）

Correction

Inhibition of cultured rat prostatic epithelial cell growth by epristeride *in vitro* (2001; 22: 257 – 263). On page 259, the abscissa should be epristeride/$\mu g \cdot L^{-1}$ not epristeride/$mg \cdot L^{-1}$ as printed. Authors are sorry for making such a mistake and puzzling readers.