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Quaternary ammonium salt derivative of haloperidol inhibits KCl-induced calcium increase in rat aortic smooth muscle cells[1]

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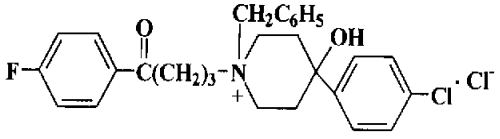
KEY WORDS calcium; confocal microscopy; haloperidol; quaternary ammonium salt; vascular smooth muscle; cultured cells

ABSTRACT

AIM: To study the effect of quaternary ammonium salt derivative of haloperidol (F_3) on intracellular calcium (Ca^{2+}) dynamic change in vascular smooth muscle cells (VSMC). **METHODS**: Using laser confocal scanning microscopy (LCSM), to observe the effect of F_3 (0.01 - 10 μmol/L) on increase of intracellular Ca^{2+} fluorescent intensity induced by KCl (30 mmol/L) in VSMC. **RESULTS**: KCl induced a rapid increase of intracellular Ca^{2+} fluorescent intensity. F_3 inhibited the rapid increase of that in both concentration-dependent manner (KCl: 67 ± 24; F_3 0.01 μmol/L: 57 ± 13; 0.1 μmol/L: 40 ± 13; 1 μmol/L: 29 ± 9; 10 μmol/L: 20 ± 6) and time-dependent manner; during 0 - 30 s after F_3 was added, the change of fluorescent intensity was more rapid. **CONCLUSION**: The inhibitory effect of F_3 on Ca^{2+} concentration in VSMC was mainly due to the blocking of the Ca^{2+} influx, and might partially attributed to opening of the potassium (K^+) channel.

INTRODUCTION

It has been reported that quaternary ammonium salt derivative of haloperidol inhibited the contraction of porcine coronary artery strips induced by KCl (30 mmol/L and 80 mmol/L) and antagonized the decrease of coronary flow on the perfusion of isolated guinea-pig heart induced by pituitary[1] in dose-dependent manner respectively, but F_3 did not result in adverse reaction of extrapyramidal system as haloperidol did[2]. In vascular smooth muscle cells, the cytosolic free Ca^{2+} concentration is a major factor, which determines the tone and activity of vascular smooth muscle. According to these experimental results, the inhibitory effects of F_3 might associate with the change of intracellular Ca^{2+} on VSMC. In present study, the laser confocal scanning microscopy (LCSM) was used to study the dynamic effects of F_3 on Ca^{2+} change in VSMC, in both dose-dependent and time-dependent manners.



F_3

MATERIALS AND METHODS

Chemicals and instruments LCSM (Tcs-NT, Leica, German), inverted microscope (Diphot-TMD, Niko, USA), CO_2 incubator (Sango, Japan), Petril dish (Gene, USA), F_3 (synthesized by chemical group of Medicine Research Department of Shantou University Medical College and analyzed by Shanghai Organic Chemistry Institute of Chinese Academy of Sciences), verapamil (Jiro Pharmaceutic, German), acetoxymethyl ester (AM) of Fluo-3 (Molecular Probe, USA), fetal bovine serum (FBS, Sijiqing, Hangzhou), $(CH_3)_2SO$ (Sigma, USA), M199 medium (Gibco, USA).

Cell culture[3] VSMC were isolated from thoratic aorta of SD rats, weighing 100 - 120 g, by outgrowth of explant method[4]. Briefly, the endothelium was carefully removed by abrasion and tunica media was peeled out from the adventitia. The smooth muscle layer segments were cut into 1 mm^2 and moved to cultural

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bottles with M199 medium, which contained 20 % FBS, penicillin (100 kU/L), and streptomycin (100 mg/L), and pH was 7.2. Cells were incubated in an incubator with 5 % CO_2. The cells in 3th[5] passage were moved to a Petri dish, and incubated for 36 h continuously, and ready for dyeing.

Cell dyeing and intracellular Ca^{2+} measurement[6] At first, the cells were washed twice with M199 solution, then incubated with 0.1 mL Fluo-3-AM 10 μmol/L for 30 min, then washed thrice again. The cells were diluted with 800 μL M199 containing Ca^{2+} (1.26 mmol/L). After the cells were loaded with Fluo-3-AM, the fluorescent intensity was measured on LCSM. According to the pre-scanning results, the proper conditions for measurement were established, *ie* excitation wavelength: 480 nm; short wavelength 530 nm; scanning type: xyt; scanning density: 512 × 512 × 60; laser power: 30 mW; PMT: 750; time interval: 5 s; scanning images: 5 min; successively. In the image, the gray value is mean fluorescence intensity for every non-zero pixel within cells, and it stands for the relative concentration of Ca^{2+}. During the course of scaning, the drugs were added into the Petri dish after the 5th image was scanned. The change of fluorescent intensity on selected cells were recorded, and the time-effect curve was obtained on computer.

Administration of drugs F_3 and verapamil were added at peak phase of fluorescence induced by KCl in VSMC.

Data analysis All data were expressed as $\bar{x} \pm s$, and t test was used for statistical analysis.

RESULTS

Effect of $(CH_3)_2SO$ on Ca^{2+} fluorescence intensity in VSMC Before addition of any agent, the fluorescent intensity stabilized for 20 min. After M199 or $(CH_3)_2SO$ were added to the Petri dish, no change of fluorescent intensity was observed.

Effect of KCl and $(CH_3)_2SO$ on Ca^{2+} fluorescent intensity in VSMC After KCl (30 mmol/L) was added to the dish, a rapid increase of Ca^{2+} fluorescent intensity was observed, and the peak phase reached at 30 s. The peak level was sustained for 20 min. Ca^{2+} fluorescent intensity was 76 ± 27, 75 ± 27, 75 ± 27, 74 ± 27, 70 ± 24, and 67 ± 24 at 0 s, 30 s, 60 s, 90 s, 120 s, and 180 s after $(CH_3)_2SO$ was added, respectively. It did not change obviously ($P > 0.05$).

Effect of F_3 (0.01 μmol/L – 10 μmol/L) and verapamil (10 μmol/L) on fluorescent intensity in VSMC After verapamil 10 μmol/L was added at the peak of fluorescent intensity in VSMC induced by KCl 30 mmol/L, the fluorescent intensity decreased rapidly, and it decreased more quickly in the period of 0 – 60 s after administration (73 ± 10, 51 ± 14, 41 ± 10, 35 ± 9, 32 ± 9, and 30 ± 9 at 0 s, 30 s, 60 s, 90 s, 120 s, and 180 s, respectively). About 3 min later, it recovered back to the level before administration of KCl.

After F_3 0.01, 0.1, 1, and 10 μmol/L were added respectively, the fluorescent intensity of VSMC induced by KCl 30 mmol/L decreased rapidly. The effect of F_3 1 μmol/L, including both change rate and effect course (Fig 1), was almost the same as that of verpamil, and showed a concentration-dependent manner and time-dependent manner for each dose (Fig 2).

DISCUSSION

In our previous study, the results showed that F_3 inhibited the contraction of coronary artery strips induced by KCl[1]. In other pre-experiment of patch clamp of smooth muscle cells, the result showed that F_3 might increase the outward K^+ currents in whole-cell record. The present study showed that F_3 decreased the Ca^{2+} concentration rapidly in VSMC. This study suggested that the effect of F_3 partially attributed to opening of K^+ channels, which might result in the cell membrane hyperpolarization that reduced the possibility of opening of voltage-dependent Ca^{2+} channels[7]. However, the major effect of F_3 is to block Ca^{2+} channels directly, because potassium channel openers can not inhibit the contraction of smooth muscle induced by KCl at a larger dose such as 80 mmol/L[8]. We consider the effect of F_3 may include dual actions, *ie*, both blocking Ca^{2+} channel and opening K^+ channel. Further studies need to be done to determine the exact mechanism of the effect of F_3 on decreasing intracellular Ca^{2+} concentration.

The results showed that intracellular Ca^{2+} fluorescent intensity changed very quickly after F_3 was added. The maximum decrease was between 0 s – 30 s, then the change curve gradually became smooth 30 s later. This indicated further that Ca^{2+}, as signal medium of cells to regulate the contraction of muscle, responsed to stimulants sensitively[9], so, it is very important to set up a proper scan time and adjust the interval time in LCSM measurement.

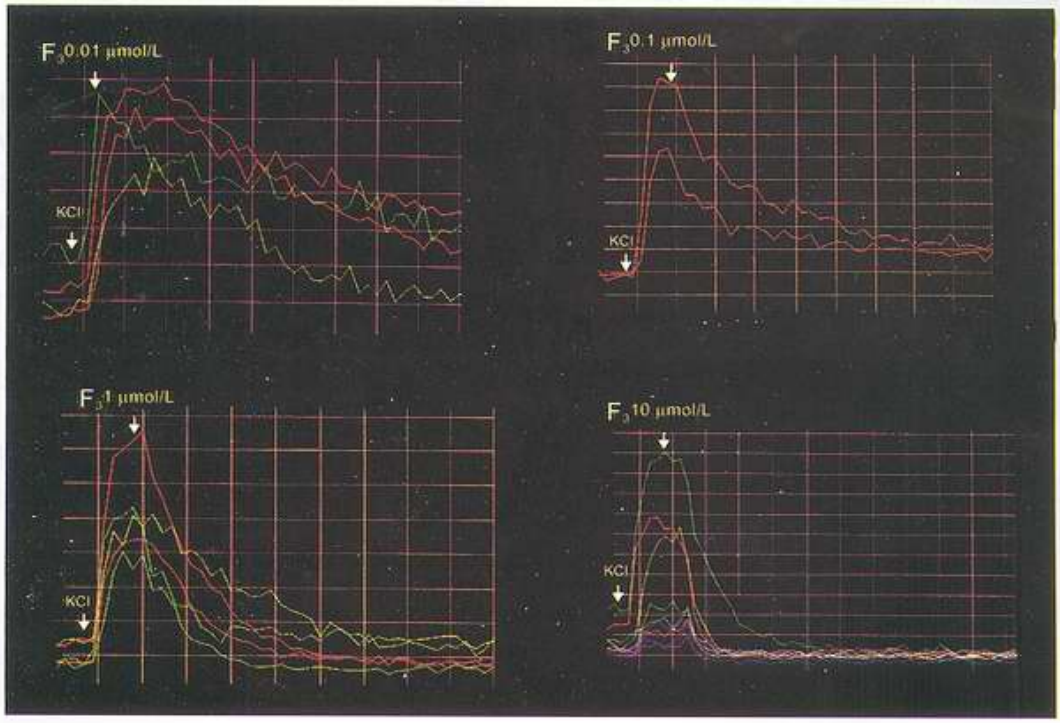

Fig 1. Effect of F_3 (0.01 – 10 μmol/L) on intracellular Ca^{2+} fluorescence intensity by KCl 30 mmol/L in rat aortic smooth muscle cells.

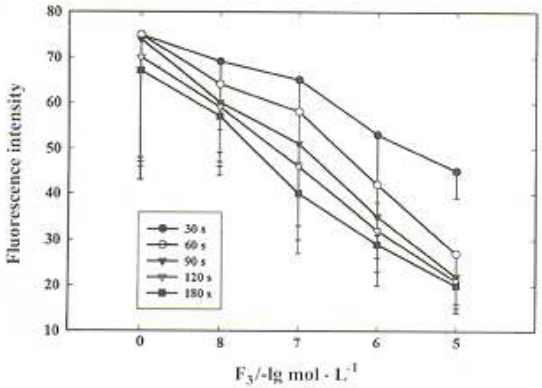

Fig 2. Concentration-dependent effect of F_3 (0.01 – 10 μmol/L) on intracellular Ca^{2+} fluorescence intensity induced by KCl 30 mmol/L in rat aortic smooth muscle cells.

In conclusion, chemical compounds that not only possess cardiovascular activities such as both Ca^{2+} channel blockers and K^+ channel openers[10,11], but also have the same structure as F_3 was not reported. According to present results, it is worthwhile to go on the research and exploitation.

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氟哌啶醇季铵盐衍生物抑制氯化钾所致大鼠主动脉平滑肌细胞内钙浓度增加[1]

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关键词　钙；共聚焦显微镜检查；氟哌啶醇；季铵盐；血管平滑肌；培养的细胞

目的：动态观察氟哌啶醇季铵盐衍生物(F_3)对血管平滑肌细胞内钙浓度的影响．**方法**：利用激光共聚焦显微镜动态观察 F_3(0.01 - 10 μmol/L)对由 KCl (30 mmol/L)诱导的大鼠血管平滑肌细胞钙荧光强度增加的作用．**结果**：KCl 可诱发细胞内钙荧光强度迅速增强，F_3 可以拮抗由 KCl 诱导的细胞内钙荧光强度增强作用，并呈量效依赖性和时间依赖性，终强度(KCl：67 ± 24；F_3 0.01 μmol/L：57 ± 13；0.1 μmol/L：40 ± 13；1 μmol/L：29 ± 9；10 μmol/L：20 ± 6)．在加入 F_3 后，钙荧光强度的变化最快时程是在给 F_3 后 0 - 30 s．**结论**：F_3 拮抗血管收缩主要是由于阻断了 Ca^{2+} 通道．

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