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# Inhibitory effect of potassium channel openers on proliferation of cultured rabbit aortic smooth muscle cells[1]

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**KEY WORDS** vascular smooth muscle; thoracic aorta; cultured cells; cell division; phenylephrine; potassium channels

**AIM:** To study the effects of pinacidil (Pin), nicorandil (Nic), RP 49356 (RP), and lemakalim (Lem) on phenylephrine (PE)-induced proliferation of cultured rabbit aortic smooth muscle cells (ASMC). **METHODS:** The [$^3$H]thymidine incorporation into DNA and number of ASMC were measured. **RESULTS:** [$^3$H]Thymidine uptake induced by PE was markedly inhibited by Pin, Nic, RP, and Lem (0.1 - 10 μmol·L$^{-1}$). Only RP inhibited [$^3$H]thymidine uptake without PE-pretreatment. Cell growth induced by PE was completely inhibited by Pin. **CONCLUSION:** Pin, Nic, RP, and Lem showed potent anti-proliferation of ASMC.

The proliferation of aortic smooth muscle cells (ASMC) is a major risk factor relevant to cardiovascular diseases. Few antihypertensive drugs exhibit antiproliferation efficiency[1,2]. Potassium channel openers (PCO) share the common property of smooth muscle relaxation and were originally developed as antihypertensive agents by hyperpolarizing the plasma membrane[3]. Minoxidil, a subtype of PCO, had no anti-proliferation but even enhanced proliferation of ASMC[4]. The inhibitory effect of pinacidil (Pin) on the increased cardiac mass was not sure[5,6]. The aim of this study is to investigate whether PCO including Pin, nicorandil (Nic), RP 49356 (RP), and lemakalim (Lem) can affect cell proliferation of cultured rabbit ASMC.

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## MATERIALS AND METHODS

**Reagents** Phenylephrine (PE), trypsin, prazosin hydrochloride, edetic acid were from Sigma Chemical Co. [$^3$H] Thymidine was from Shanghai Institute of Nuclear Research. Pin, Nic, RP, Lem, and glibenclamide (Gli) were gifts from Leo Pharmaceutical Products Ltd (Denmark), Fuzhou Military Medical School, Centre de Recherche de Vitro-Alfortville (France), SmithKline Beecham Pharmaceuticals (UK), and Tianjin Institute of Pharmaceutical Industries, respectively. Pin, Lem, RP, and Gli were dissolved in 0.001 % $Me_2SO$; other agents were dissolved in distilled water.

**Cell culture** ASMC were isolated from the thoracic aorta of 8-week-old rabbit by outgrowth of the explants method[7]. Cells in 8th to 10th passage were used in the experiment.

**[$^3$H] Thymidine incorporation into DNA[8]** ASMC were seeded at $1 \times 10^4$ cells/well into 24 well plastic plates by trypsinization. RPMI 1640 medium (Gibco) with 10 % newborn calf serum (NCS) was refreshed every other day. After 4 d, cell synchronization was initiated by serum-free medium culturing for 72 h. The plated ASMC was stimulated again with 10 % NCS and the 4 PCO (0.1 - 10 μmol·L$^{-1}$) or prazosin (10 μmol·L$^{-1}$) combined with PE (10 μmol·L$^{-1}$) were added for another 36 h culturing. PE (10 μmol·L$^{-1}$) and 4 PCO (1 μmol·L$^{-1}$) were tested alone at the same conditions. Then [$^3$H]thymidine 37 MBq·L$^{-1}$ was added. After incubation for another 6 h, cells were washed twice with 0.9 % NaCl and harvested by dispersing with 0.2 % trypsin solution and transferred on the filter paper, then treated with ice-cold 10 % trichloroacetic acid for 10 min and washed with 0.9 % NaCl again. The filter paper was dried and placed into the scintillation solution (PPO 5 g, POPOP 0.2 g in 1 L xylene). The radioactivity was measured by a liquid scintillation counter (Beckman LS3801, USA).

**Cell growth** The confluent culture of ASMC was seeded at $1 \times 10^4$ cells/well into 24 well plastic plates by trypsinization. Cell synchronization was initiated. The medium was supplemented with 10 % NCS containing vehicle (0.001 % $Me_2SO$) or Pin (10 μmol·L$^{-1}$) with or without PE (10 μmol·L$^{-1}$). The medium was changed every other day. After 4 d, the number of VSMC was determined using

a hemocytometer after trypsinization.

**Statistical analysis** The $t$ test and ANOVA were used.

## RESULTS

**Effect of PCO on [$^3$H] thymidine uptake** When treated with 0.001 % $Me_2SO$ as control, only RP (1 μmol·L$^{-1}$) possessed inhibitory effect of [$^3$H]thymidine uptake [$(1.37 \pm 0.26) \times 10^3$ *vs* $(12.6 \pm 1.0) \times 10^3$ dpm/well, $P<0.01$, Tab 1)]. However, [$^3$H] thymidine incorporation into DNA in ASMC pre-stimulated by PE 10 μmol·L$^{-1}$ was inhibited by the 4 PCO 0.1 – 10 μmol·L$^{-1}$. The inhibitory effect of these PCO, except for Pin, were stronger than that of prazosin [$(24.8 \pm 1.4) \times 10^3$ dpm/well] on PE-induced [$^3$H]thymidine uptake in ASMC at 10 μmol·L$^{-1}$ ($P<0.01$, Fig 1).

**Tab 1. Effects of the four potassium channel openers on [$^3$H] thymidine uptake and cell number in cultured rabbit aortic smooth muscle cells.** $n = 6$ **wells.** $\bar{x} \pm s$. $^aP > 0.05$. $^cP<0.01$ *vs* **control (0.001 % $Me_2SO$);** $^fP<0.01$ *vs* **PE group.**

| | [$^3$H]Thymidine uptake ($10^{-3}$ · dpm/well) | Cell number ($10^{-3}$ · cells/well) |
|---|---|---|
| Control | 12.6 ± 1.0 | 12.4 ± 1.7 |
| PE (10 μmol·L$^{-1}$) | 68 ± 12$^c$ | 16.3 ± 1.4$^c$ |
| Pin (10 μmol·L$^{-1}$) | – | 11.2 ± 1.3$^a$ |
| PE + Pin (10 μmol·L$^{-1}$) | – | 10.5 ± 1.8$^{af}$ |
| Pin (1 μmol·L$^{-1}$) | 13.8 ± 1.0$^a$ | – |
| Nic (1 μmol·L$^{-1}$) | 14.2 ± 1.6$^a$ | – |
| RP (1 μmol·L$^{-1}$) | 1.37 ± 0.26$^c$ | – |
| Lem (1 μmol·L$^{-1}$) | 11.2 ± 1.6$^a$ | – |

**Role of Pin in ASMC growth** The ASMC increase induced by PE (10 μmol · L$^{-1}$) was completely inhibited by Pin (Tab 1).

The morphological appearances of the ASMC in cell size, shape, size ratio of nucleus/cell were not changed under microscopy and electron microscopy.

## DISCUSSION

Over-proliferation of smooth muscle cells in blood vessel walls plays an important role both in the perpetuation and in the initiation of the hypertensive process[4]. A variety of growth factors such as noradrenaline and its analogues can stimulate ASMC proliferation. PE-induced mitosis of ASMC is due

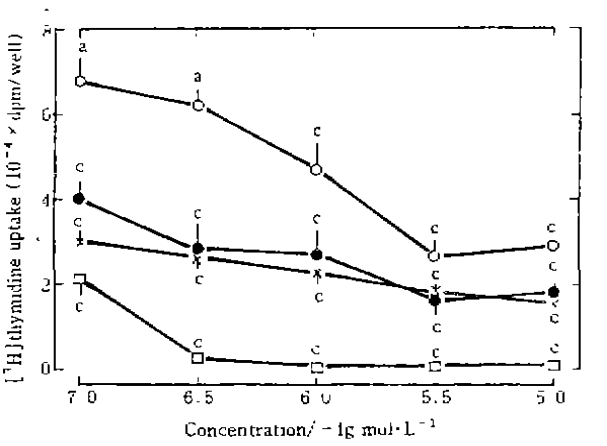


**Fig 1. Inhibition of $K^+$ channel openers on inhibiting PE (10 μmol·L$^{-1}$)-induced [$^3$H]thymidine uptake of cultured rabbit aortic smooth muscle cells.** $n = 6$ **wells,** $\bar{x} \pm s$. $^aP>0.05$, $^cP<0.01$ *vs* **control [0.001 % $Me_2SO$ + PE, by $(68 \pm 12) \times 10^3$ dpm/well].**

to increasing calcium influx[9].

PCO have potential in the treatment of hypertension. The mechanism of their actions is attributed to the opening of potassium channel and the consequent hyperpolarizing of the plasma membrane. Membranes hyperpolarization can result in active $Ca^{2+}$ extrusion or inhibit the refilling of intracellular $Ca^{2+}$ stores[3,10]. Our previous experiment also confirmed that these 4 PCO lowered the cytosolic free calcium on ASMC[7]. Therefore, it is suggested that the mechanism of antiproliferation of 4 PCO on PE-induced ASMC growth is associated with lowering $[Ca^{2+}]_i$.

However, there are seven ATP-sensitive subtypes of PCO. Meisheri *et al*[11] indicated that minoxidil must be active to form minoxidil sulfate *in vivo*. Sulfate moiety from minoxidil sulfate play a key role in vasorelaxation. In heart, pinacidil is generally effective at concentrations several orders of magnitude greater than that evoking vasodilation[12]. In addition, cell edema is a common adverse effect of Pin for antihypertension[13]. These might be the explanation of the uncertain effect of Pin on increased cardiac mass. At the present study, why only RP can inhibit [$^3$H] thymidine incorporation into ASMC without PE stimulation and whether the antiproliferation effects and their mechanisms of the 4 PCO are associated their different structure, further investigation is to

be needed.

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## 钾通道开放剂对培养兔主动脉平滑肌细胞增殖的抑制作用[1]

R972
R965

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**关键词** 血管平滑肌；胸主动脉；培养的细胞；细胞分裂；苯福林；钾通道

**目的**：探讨吡那地尔（Pin），尼可地尔（Nic），RP 49356（RP）及雷马克林（Lem）对苯福林（PE）诱导的培养兔胸主动脉平滑肌细胞（ASMC）增殖的影响. **方法**：测定[$^3$H]胸腺嘧啶掺入 ASMC DNA 的放射活性及细胞数目. **结果**：Pin，Nic，PR 及 Lem 对 PE 诱导的[$^3$H]胸腺嘧啶摄取具有明显的抑制作用. 对正常细胞的生长，仅 RP 具有抑制[$^3$H]胸腺嘧啶摄取的作用 在 Pin 组，PE 诱导的细胞数目增加被完全抑制 **结论**：Pin，Nic，RP 及 Lem 具有抑制 ASMC 增殖的作用