

## Inhibition of myocardial inward rectifier potassium current by propylbutyldopamine

DUN Wen, ZHAO Rong-Rui<sup>1</sup>, LI Jian-Guo, WU Bo-Wei

(Department of Physiology, Shanxi Medical University, Taiyuan 030001, China)

**KEY WORDS** propylbutyldopamine; domperidone; myocardium; potassium channels; calcium channels; action potentials; patch-clamp techniques

### ABSTRACT

**AIM:** To study the effects of propylbutyldopamine (PBDA) on the inward rectifier potassium current ( $I_{K1}$ ). **METHODS:** The quasi-steady state current-voltage relationship from the isolated guinea pig ventricular cells were measured using whole-cell patch-clamp techniques with a slow ramp depolarization ( $8 \text{ mV} \cdot \text{s}^{-1}$ ). **RESULTS:** PBDA 5, 50, and  $100 \mu\text{mol} \cdot \text{L}^{-1}$  concentration-dependently reduced the inward rectifier potassium current. PBDA blocked  $I_{K1}$  in guinea pig ventricular cells. The effect of PBDA was not blocked by the selective dopamine  $D_2$ -receptor blocker, domperidone. **CONCLUSION:** PBDA inhibited  $I_{K1}$  directly, independent of the dopamine  $D_2$ -receptor.

### INTRODUCTION

Propylbutyldopamine (PBDA), a selective dopamine  $D_2$ -receptor agonist, decreased blood pressure and heart rate primarily through activation of specific dopamine  $D_2$ -receptors located in the peripheral rather than central nervous system in anesthetized dogs<sup>[1]</sup>. In patients with congestive heart failure, PBDA reduced mean arterial pressure, left ventricular filling pressure, pulmonary and systemic vascular resistances, and increased cardiac index, without changes in either stroke work index or heart rate<sup>[2]</sup>. Compared with

fenoldopamine, PBDA never induced ventricular arrhythmias<sup>[3]</sup>. Because of this potential improved efficacy of heart function, a better understanding of ionic mechanisms underlying the cardiac actions of PBDA is essential. Thus, we assessed the myocardial electrophysiologic properties of PBDA. The physiological function of  $D_2$  receptor in heart is not clear as yet. Previous physiologic studies failed to demonstrate the involvement of  $D_2$  receptors in cardiac inotropic mechanisms<sup>[3]</sup>. This paper was to study the electrophysiologic effects of PBDA on the inward rectifier potassium current ( $I_{K1}$ ) to evaluate the functional significance of cardiac  $D_2$  receptors.

### MATERIALS AND METHODS

**Drugs** PBDA and domperidone were synthesized by Sloan-Kettering Cancer Center (USA) and Janssen Pharmaceutica Inc ( Piscataway, NJ, USA ), respectively. Both drugs in white powder (purity 98 %) were dissolved in distilled water at a stock concentration of  $10 \text{ mmol} \cdot \text{L}^{-1}$  which was subsequently diluted in Tyrode's solution by serial dilutions to produce final desired concentration.

**Isolation of single ventricular cells** Dunkin Hartley guinea pigs ( $n = 24$ ,  $245 \text{ g} \pm s 10 \text{ g}$ ) were provided by Experimental Animal Center of Shanxi Medical University (Grade II, Certificate No 0351095). Single ventricular myocytes were obtained by the enzymatic isolation<sup>[4]</sup>. After stabilization at  $22 - 24 \text{ }^\circ\text{C}$  for 1 h, isolated cells were placed in a recording chamber on the stage of an inverted microscope (XDP-1, Shanghai), where prewarmed Tyrode's solution was continuously superfused at a rate of  $1 - 2 \text{ mL} \cdot \text{min}^{-1}$  at  $32 \text{ }^\circ\text{C}$ . The Tyrode's solution contained  $\text{NaCl}$  139,  $\text{MgCl}_2$  1.0,  $\text{KCl}$  5.4,  $\text{CaCl}_2$  1.8,  $\text{NaH}_2\text{PO}_4$  1.0, HEPES 5.0, glucose  $10 \text{ mmol} \cdot \text{L}^{-1}$ , pH adjusted to 7.4 with NaOH.

<sup>1</sup> Correspondence to Prof ZHAO Rong-Rui.

Phn 86-351-413-5057. Fax 86-351-406-0031.

E-mail sydw@shanxi.ihp.ac.cn

Received 1998-02-26

Accepted 1998-11-18

**Electrophysiologic measurements** A conventional whole-cell 'GΩ seal' recording was used<sup>[5]</sup>. The electrodes were pulled in 2 stages by 2-step progress patch pipette puller (Narishige, Japan). The electrode resistance ranged from 2 to 3 MΩ. They were filled with the solution containing K-Asp 80, KCl 50, MgCl<sub>2</sub> 1.0, HEPES 5.0, egtazic acid 1.0, Na<sub>2</sub>-ATP 3.0 mmol · L<sup>-1</sup>, pH adjusted to 7.4 with KOH. The transmembrane voltage and currents were recorded using a patch-clamp amplifier (Axopatch 200A, Axon Instruments, USA). Computer program pCLAMP 5.5.1 (Axon Instrument) was used to produce current and voltage clamping signals. Data acquisition and analysis were performed with this software and an AST 386 computer.

Action potentials (AP) were recorded from isolated myocytes in the current clamp mode by 2-5 ms suprathreshold stimulation at stimulus frequencies of 0.2 Hz. Only cells showing normal resting potentials (-70 to -85 mV) and AP configuration were used in this study. Effects of PBDA on action potential duration (APD) were studied in control at 3-5 min after perfusion with PBDA 5, 50, and 100 μmol · L<sup>-1</sup>. A slowly depolarizing voltage ramp was used to study the effect of PBDA on the quasi-steady state I-V relations for K<sup>+</sup> currents in guinea pig myocytes. Voltage ramps were applied beginning at -100 mV, slowly depolarizing to +60 mV at a rate of 8 mV · s<sup>-1</sup> from a -80 mV holding potential. Calcium currents were evoked by depolarization of individual ventricular cells from a holding potential of -40 mV to 0 mV at 0.2 Hz.

**Statistics** Data were expressed as  $\bar{x} \pm s$  and analyzed by paired *t* test.

## RESULTS

**Effects of PBDA on APD** PBDA 5, 50, and 100 μmol · L<sup>-1</sup> caused a concentration-dependent lengthening of the action potential (Tab 1, Fig 1).

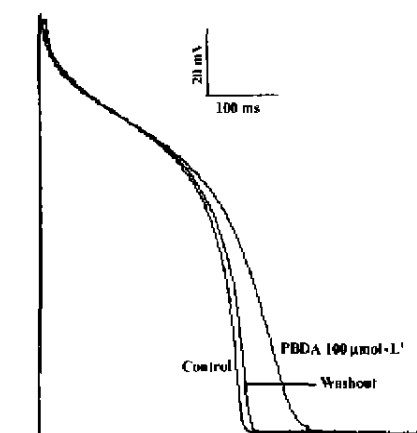
The slope conductance was decreased, consistent with the blockade of *I<sub>kl</sub>* by PBDA. At -60 mV, the current amplitudes before and during the application of PBDA 5, 50, and 100 μmol · L<sup>-1</sup> changed from (568 ± 200), (577 ± 83), and (488 ± 113) pA in control condition to (566 ± 199) (-1.1% ± 1.6%, *n* = 6, *P* < 0.05), (402 ± 85) (-31% ± 6%, *n* = 6, *P* <

0.01), and (214 ± 65) pA (-56% ± 7%, *n* = 5, *P* < 0.01) in the presence of drugs, respectively.

**Tab 1.** Effects of PBDA on the characteristics of action potentials in guinea pig ventricular cells. *n* = 6 cells from 6 guinea pigs.  $\bar{x} \pm s$ . <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs control. RMP: resting membrane potential. APD<sub>20</sub> and APD<sub>90</sub>: action potential duration at 20% and 90% repolarization.

Drug/ μmol · L <sup>-1</sup>	RMP/mV	APD <sub>20</sub> /ms	APD <sub>90</sub> /ms
Control	-76 ± 4	155 ± 44	358 ± 84
PBDA 5	-76 ± 5	156 ± 44	360 ± 82 <sup>b</sup>
Control	-76 ± 3	149 ± 54	341 ± 105
PBDA 50	-76 ± 4	149 ± 54	417 ± 118 <sup>c</sup>
Control	-77 ± 5	163 ± 60	383 ± 108
PBDA 100	-77 ± 5	163 ± 57	533 ± 129 <sup>c</sup>

**Effect of PBDA on the I-V relations of outward K<sup>+</sup> currents** The I-V curve had a characteristic "N" shape with a region of inward rectification and negative slope. After exposure to PBDA, the net current shifted in inward direction over the range of -100 to -40 mV (Fig 2).



**Fig 1.** Effects of propylbutyldopamine 100 μmol · L<sup>-1</sup> on action potentials in guinea pig ventricular myocytes. A representative recording of 6 cells from 5 guinea pigs.

### PBDA on L-type inward calcium currents

PBDA showed no effect on the net inward Ca<sup>2+</sup> currents. However, PBDA reduced current amplitude at the -40 mV holding potential, indicating that

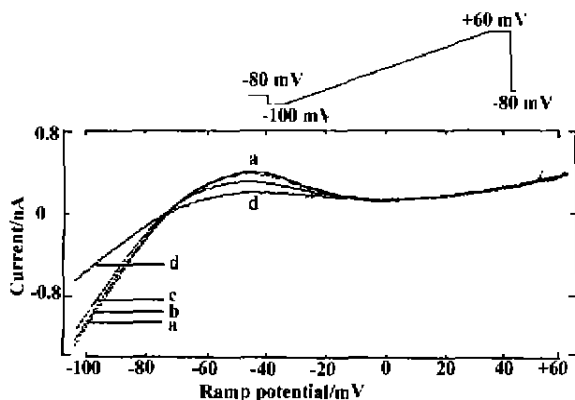


Fig 2. Concentration-dependent effects of PBDA on current-voltage relationship with a voltage ramp protocol in guinea pig ventricular myocytes. Control (a); PBDA 5 (b), 50 (c), and 100 (d)  $\mu\text{mol}\cdot\text{L}^{-1}$ . A representative recording of 6 cells from 6 guinea pigs.

PBDA inhibited the inwardly rectifying  $\text{K}^+$  current outward (Fig 3).

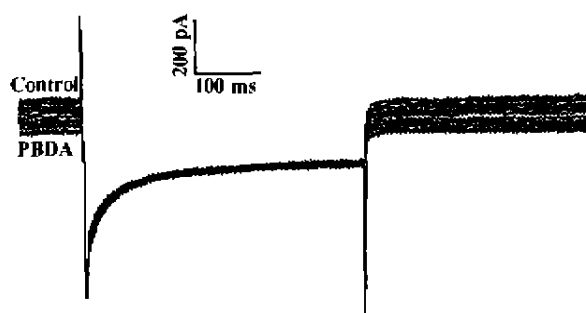


Fig 3. Action of PBDA  $100\ \mu\text{mol}\cdot\text{L}^{-1}$  on  $I_{\text{Ca}}$  in guinea pig ventricular myocytes. An original current trace of  $I_{\text{Ca}}$  change induced by PBDA. A representative recording of 6 cells from 5 guinea pigs.

**Domperidone on PBDA-induced changes of APD and  $I_{\text{K1}}$**  Domperidone  $20\ \mu\text{mol}\cdot\text{L}^{-1}$  had no effect on the changes of  $\text{APD}_{90}$  and  $I_{\text{K1}}$  induced by PBDA ( $P > 0.05$ ) (Fig 4).

## DISCUSSION

In the present experiment, PBDA prolonged  $\text{APD}_{90}$  and inhibited  $I_{\text{K1}}$ , without altering  $\text{APD}_{20}$ .  $I_{\text{K1}}$  mainly contributes to the final phase of rapid repolarization at the end of action potential<sup>[6]</sup>. The

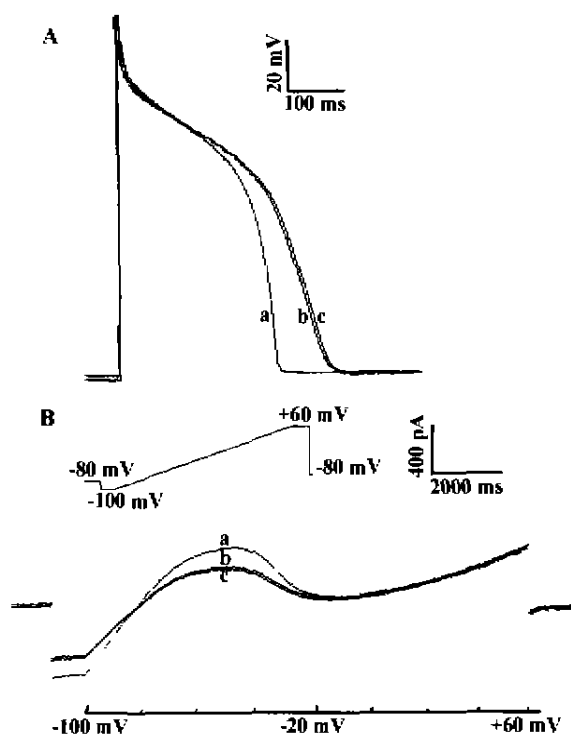


Fig 4. Effects of domperidone on PBDA-induced changes in action potentials and  $I_{\text{K1}}$  in guinea pig ventricular myocytes.  $n = 6$  cells from 6 guinea pigs. A) Action potentials before (a) and after (b) exposure to PBDA  $100\ \mu\text{mol}\cdot\text{L}^{-1}$ . B)  $I_{\text{K1}}$  with a voltage-ramp protocol before (a) and after (b) exposure to PBDA  $100\ \mu\text{mol}\cdot\text{L}^{-1}$  and PBDA  $100\ \mu\text{mol}\cdot\text{L}^{-1}$  + domperidone (c).

PBDA-induced inhibition of  $I_{\text{K1}}$  satisfactorily accounted for the prolongation of  $\text{APD}_{90}$  but no changes of  $\text{APD}_{20}$  caused by PBDA. To study the possible mechanisms underlying the effects of PBDA, we used domperidone, a highly selective dopamine  $\text{D}_2$ -receptor antagonist to block the  $\text{D}_2$  receptors and to see if the effects of PBDA can be abolished. Contrary to our expectation, domperidone showed no blockade effect on the changes of PBDA-induced  $\text{APD}_{90}$  and  $I_{\text{K1}}$ . Thus, we demonstrated, for the first time, that PBDA manifested its blockade effect on inward rectifier  $\text{K}^+$  channels independently of dopamine  $\text{D}_2$ -receptors. Therefore, PBDA *per se* might have a direct effect on myocyte  $I_{\text{K1}}$  channel. However, the study on PBDA is still preliminary. Further experiments are needed to confirm that PBDA is a new  $I_{\text{K1}}$  blocker.

In this study, our findings also suggest that

stimulation of cardiac  $D_2$  receptors had no effect on the electrophysiological properties of the heart. Therefore, the functional significance of cardiac  $D_2$  receptors in the heart, if any, still remains unresolved.

nomenclature, properties, function and cloned equivalents. Cardiovasc Res 1996; 32: 455-81.

## REFERENCES

- 1 Taylor AA, Fennell WH, Feldman MB, Brandon TA, Ginos JI, Mitchell JZ. Activation of peripheral dopamine presynaptic receptors lowers blood pressure and heart rate in dogs. *Hypertension* 1983; 5: 226-34.
- 2 Fennell WH, Taylor AA, Young JB, Brandon TA, Ginos JZ, Goldberg LI, *et al.* Propylbutyldopamine: hemodynamic effects in conscious dogs, normal human volunteers and patients with heart failure. *Circulation* 1983; 67: 829-36.
- 3 Zhao RR, Fennell WH, Abel FL. Effects of dopamine  $D_1$  and dopamine  $D_2$  receptor agonists on coronary and peripheral hemodynamics. *Eur J Pharmacol* 1990; 190: 193-202.
- 4 Isenberg G, Klöckner U. Calcium tolerant ventricular myocytes prepared by preincubation in a "KB medium". *Pflügers Arch* 1982; 395: 6-18.
- 5 Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 1981; 391: 85-100.
- 6 Boyett MR, Harrison SM, Janvier NC, McMom SO, Owen JM, Shui Z. A list of vertebrate cardiac ionic currents;

## 丙基丁基多巴胺对心肌内向整流钾电流的抑制作用

顿文, 赵荣瑞<sup>1</sup>, 李建国, 吴博威  
(山西医科大学生理教研室, 太原 030001, 中国)

**关键词** 丙基丁基多巴胺; 多潘立酮; 心肌; 钾通道; 钙通道; 动作电位; 膜片钳技术

**目的:** 研究丙基丁基多巴胺(PBDA)对内向整流钾电流( $I_{K1}$ )的影响。 **方法:** 应用缓慢斜坡去极化脉冲程序测定离体豚鼠心室肌细胞准稳态电流-电压关系曲线。 **结果:** PBDA 5, 50 和 100  $\mu\text{mol}\cdot\text{L}^{-1}$  浓度依赖性地降低了心肌内向整流钾电流, 表明该药对  $I_{K1}$  具有抑制作用。 PBDA 对于  $I_{K1}$  的这种抑制效应不被多巴胺  $D_2$  受体选择性阻断剂多潘立酮所阻断。 **结论:** PBDA 直接抑制  $I_{K1}$ , 与多巴胺  $D_2$  受体无关。

(责任编辑 杨雪芳)