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Blockade of L-type calcium channel in myocardium and calcium-induced contractions of vascular smooth muscle by CPU 86017¹

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KEY WORDS 4-chlorobenzyltetrahydroberberine; myocardium; vascular smooth muscle; patch-clamp techniques; berberine; calcium

ABSTRACT

AIM: To assess the blockade by CPU 86017 on the L-type calcium channels in the myocardium and on the Ca²⁺-related contractions of vascular smooth muscle. **METHODS:** The whole-cell patch-clamp was applied to investigate the blocking effect of CPU 86017 on the L-type calcium current in isolated guinea pig myocytes and contractions by KCl or phenylephrine (Phe) of the isolated rat tail arteries were measured. **RESULTS:** Suppression of the L-type current of the isolated myocytes by CPU 86017 was moderate, in time- and concentration-dependent manner and with no influence on the activation and inactivation curves. The IC₅₀ was 11.5 μmol/L. Suppressive effect of CPU 86017 on vaso-contractions induced by KCl 100 mmol/L, phenylephrine 1 μmol/L in KH solution (phase 1), Ca²⁺ free KH solution (phase 2), and by addition of CaCl₂ into Ca²⁺-free KH solution (phase 3) were observed. The IC₅₀ to suppress vaso-contractions by calcium entry via the receptor operated channel (ROC) and voltage-dependent channel (VDC) was 0.324 μmol/L and 16.3 μmol/L, respectively. The relative potency of CPU 86017 to suppress vascular tone by Ca²⁺ entry through ROC and VDC is 1/187 of prazosin and 1/37 of verapamil, respectively. **CONCLUSION:** The blocking effects of CPU 86017 on the L-type calcium channel of myocardium and vessel are moderate and non-selective. CPU 86017 is approximately 50 times more potent in inhibiting ROC than VDC.

INTRODUCTION

CPU 86017 (*p*-chloro-benzyl-tetrahydroberberine), a derivative of berberine, blocked multiple ion channels evaluated by standard microelectrode electrophysiological study^[1] and patch-clamp techniques. CPU

86017 is produced by attaching *p*-chlor-benzyl-side chain and hydrogenation on the moiety of berberine^[2,3]. The bioavailability of berberine is very poor. So modification of moiety of berberine will be helpful to improve the solubility and bioavailability of berberine for oral medication. The potency of CPU 86017 was 7 times stronger than berberine to suppress the ouabain-induced arrhythmias in guinea pigs^[4]. The inhibitory effects of CPU 86017 on phenylephrine induced contractions of rat anococcygeus had no significant difference compared with berberine^[5]. The potency of CPU 86017 to suppress contractions of rat thoracic aortic ring induced by high K⁺ in normal KH solution is as 3 times as ber-

¹ Supported by grant from the National Natural Science Foundation of China, No 39670835 & 30230170.

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Received 2002-12-31

Accepted 2003-10-14

berine^[6]. However the capability of CPU 86017 to suppress the contractions via the voltage dependent channel (VDC) is 10 time more potent than berberine^[4]. It is implied that the L-type channels in myocardium was blocked by CPU 86017. The L-type Ca^{2+} channels in myocytes of guinea pigs are enhanced by chronic medication of L-thyroxin^[7] and suppressed by propranolol^[8]. CPU 86017 suppresses the electrical activity of pacing cells in the sino-atrial node^[1] where the I_{Ca} is the main inward current to depolarize membrane and the developed force for atrial and papillary muscle contraction^[5]. A reduction in heart rate and blood pressure after CPU 86017 intravenous treatment were observed^[7,8].

The present study aimed to confirm the inhibitory effect of CPU 86017 on I_{Ca} current in isolated guinea-pig myocytes and to determine whether its inhibitory effects on Ca^{2+} -related contractions of the rat tail artery.

MATERIALS AND METHODS

Animals and chemical Guinea pigs 250-300 g, either sex, were provided by the Animal House of the Life Science Institute. SD rats, Grade II of either sex, 200±12 g, were supplied by the Experimental Animal Center of China Pharmaceutical University. The compound CPU 86017 was synthesized by the Center of New Drug Research of China Pharmaceutical University (Fig 1).

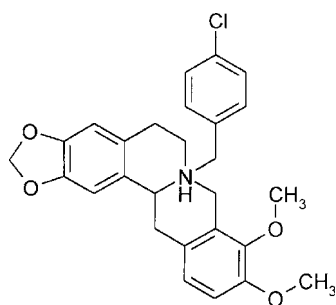


Fig 1. The chemical structure of CPU 86017.

Isolation of myocytes from guinea pig heart^[7,8]

In brief, the heart was taken quickly after a blow at the head, and mounted and infused at the Langendorff's apparatus at 37 °C with O_2 gassed in series of solutions. The perfusion with the solution A was made at 6 mL/min for 5 min and followed by perfusion of an enzyme containing solution for 3 min to soften the heart. The ventricle was cut into small pieces at a size of 1 mm³ at 37 °C in the solution B for 3-5 min, then, the precipitate

was moved into an enzyme containing solution B for 5 min. The supernatant was added with solution C 1.5 mL and striated myocytes with rod shape were selected for whole-cell patch-clamp to determine the I_{Ca} currents.

Reagents and solutions for patch clamp (mmol/L): 1) Solution A: NaCl 116, KCl 5.4, NaH_2PO_4 1.4, MgSO_4 1.2, NaHCO_3 15, glucose 15, pH adjusted to 7.4 by NaOH. 2) Solution B: NaCl 116, KCl 5.4, NaH_2PO_4 1.4, MgSO_4 1.2, CaCl_2 0.15, NaHCO_3 15, glucose 15, XIV type protease 0.1 g/L, BSA 1 g/L. 3) Solution C: NaCl 116, KCl 5.4, NaH_2PO_4 1.4, MgSO_4 1.2, CaCl_2 0.5, NaHCO_3 15, glucose 15, XIV type protease 0.1 g/L, BSA 0.5 g/L. 4) The electrode solution: CsCl 140, MgCl_2 2, CaCl_2 , egtazic acid 11, Na-ATP 5, HEPES 10. 5) The cell bath solution: NaCl 116, KCl 5.4, NaH_2PO_4 1.4, MgSO_4 1.2, CaCl_2 0.5, NaHCO_3 15, glucose, pH adjusted to 7.4 with NaOH.

Pronase E, HEPES, TEA-Cl, 4-AP, egtazic acid, Na-ATP, Tris, XIV protease, and BSA were products of Sigma. Tetrodotoxin (TTX) was from the Beijing Institution of Chemical Protection.

Whole-cell patch-clamp The patch clamp was conducted with the following instruments: the inverse microscopy (Nikon 810185, Japan), the microelectrode controller (Narishige PP-83, Japan), the liquid pressed processor (Narishige MD-320, Japan), the patch-clamp amplifier (EPC-7, List-Medical, Germany), the DMA sealing system (Labmaster Model TL-1, Axon Instruments, USA), computer (Casper, PC-386/IBM, USA) were used.

The CsCl electrode was applied to hold the myocyte at -40 mV and the stimulate potential was set from -40 mV up to +50 mV with an interval of 10 mV. Under this condition the inward I_{Na} and the T-type current were inactivated and the potassium current was suppressed by CsCl^[11].

Contractions of rat tail artery The tail artery was carefully dissected and 3-mm length ring was mounted in 3 mL organ bath with a load of 0.6 g. The tail artery was treated with norepinephrine 1 $\mu\text{mol/L}$ twice to stabilize the arterial contractile activity and washed and balanced for approximately 2 h. The contraction induced by phenylephrine 1 $\mu\text{mol/L}$ or KCl 100 mmol/L in three phases: in the normal KH solution (phase 1), the Ca^{2+} free KH solution (phase 2), and after addition of Ca^{2+} into the Ca^{2+} free medium (phase 3), respectively^[12,13].

The IC_{50} of CPU 86017 on the contractions in the

three phases was determined separately in comparison with positive reference drug prazosin (Sigma) and verapamil (Lianyungang Pharmaceutical Factory) to show the potency to suppress intracellular Ca^{2+} release (phase 2), the Ca^{2+} channels of receptor operated channel (ROC) and voltage-dependent channel (VOC) (phase 3) and the mixed action (phase 1), functionally and respectively^[12,13]. The constituents of KH solution and the Ca^{2+} free KH solution were as the previous description^[12].

Statistic analysis The Student *t*-test was applied to test the difference between groups and the statistic significance was set at $P < 0.05$ and $P < 0.01$.

RESULTS

The L-type currents of isolated myocytes from guinea pigs An inward L-type Ca^{2+} current was re-

corded when the holding potential was set at the -45 mV to block the I_{Na} and 4-AP and CsCl were added to block the I_{to} and I_{K} , separately. An inward current which showed its maximal current at 0 mV and was suppressed completely by 4-min exposure to verapamil 1 $\mu\text{mol/L}$ and recovered to a large extent after washing (data not shown). It was recognized and confirmed as the L-type Ca^{2+} current. The run-down phenomena of the L-type current was observed at maximum by 25 % in the first 20 min and the suppressive effect of CPU 86017 was observed during matched period and the run down was cleared (Fig 2).

CPU 86017 suppressed Ca^{2+} current in a time-dependent manner CPU 86017 10 $\mu\text{mol/L}$ caused a reduction of the Ca^{2+} current at 6 min and further reduction at 12 min. The suppression was only partially recovered after washing (Fig 2A).

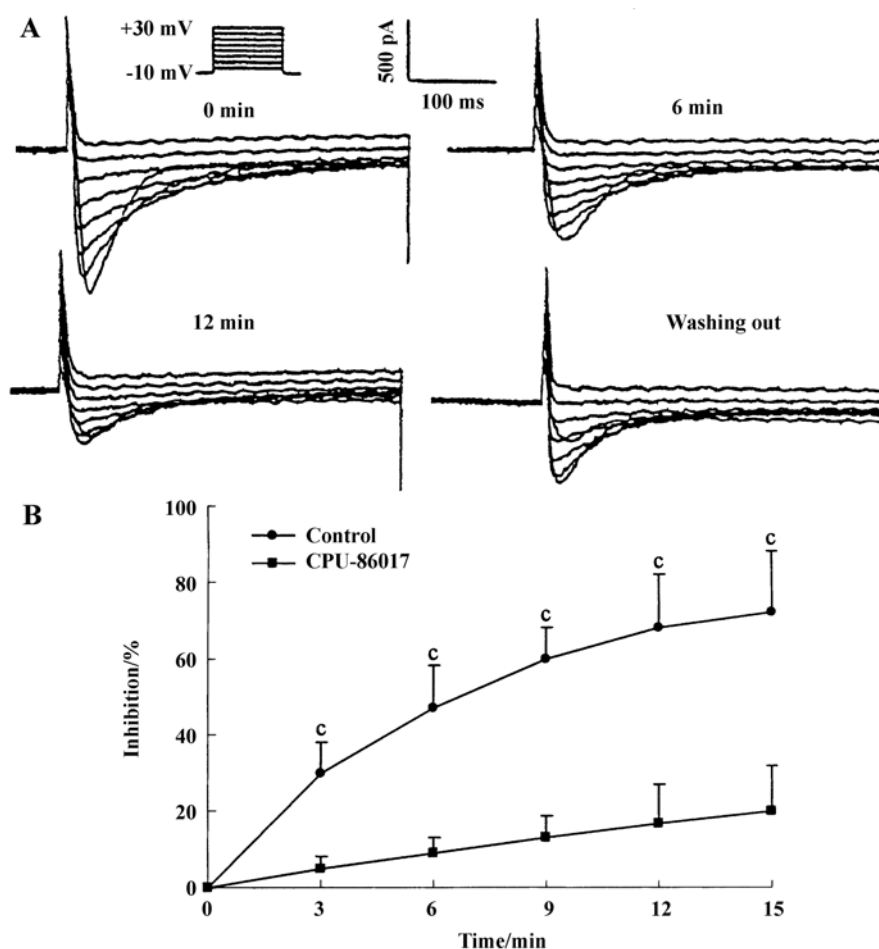


Fig 2. Effect of CPU 86017 10 $\mu\text{mol/L}$ on L-type calcium current in ventricular myocytes of guinea pig. **A)** Calcium currents were elicited by the voltage protocol illustrated in the inset. Current traces are shown before (0 min) and after application of CPU 86017 at 6 min, 12 min and washing out. **B)** Time dependent effect of I_{Ca} blockade by CPU 86017. Percent blockade is determined and voltage step to 0 mV, according to the formula $100 \times (1 - I_a/I_b)$, where I_b and I_a are the current amplitude before and after application of CPU 86017, respectively. $n=4-5$ from 4 guinea pigs. $^c P < 0.01$ vs control.

In another experiment after exposure to CPU 86017 suppression was initiated at 1-2 min, and the inhibition on the peak current at the 0 mV was enhanced along with time and reached the maximum at 15 min (Fig 2B) with half blocking time of (7.2 ± 2.1) min.

CPU 86017 suppressed L-type Ca^{2+} current in a concentration-dependent manner CPU 86017 1, 3.3, 10, and 33 $\mu\text{mol/L}$ suppressed L-type Ca^{2+} current by $20\% \pm 11\%$, $37\% \pm 8\%$, $66\% \pm 6\%$, and $79\% \pm 3\%$ at 15 min, respectively (Fig 3B). The IC_{50} value was $11.5 \mu\text{mol/L}$.

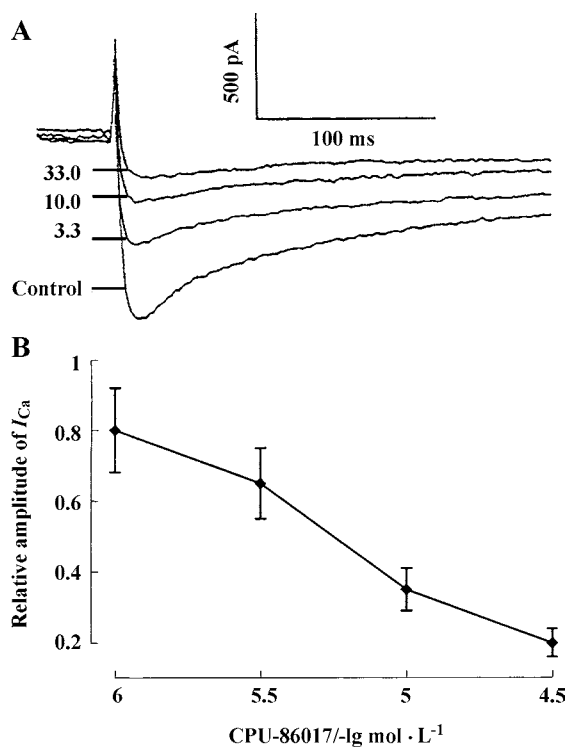


Fig 3. Effects of CPU 86017 3.3-33 $\mu\text{mol/L}$ on L-type calcium currents. A) Ca^{2+} currents elicited by a test pulse to 0 mV from HP of -40 mV. B) CPU 86017 inhibited L-type current in a concentration-dependent manner. $n=4\sim 5$ cells from 4 animals. Mean \pm SD. (subtraction of run down).

In voltage-current relationship the suppression by CPU 86017 reached the maximum at the 0 mV. CPU 86017 shifted the I - V curve upward but did not change the shape (Fig 4).

CPU 86017 had no effect on activation and inactivation curve of L-type calcium current Taking the maximal current as a unity the plot of I/I_{max} against potential yielded a stable activation curve which was fitted with the Boltzmann equation: $I/I_{\text{max}} = 1 / \{1 + \exp[-(V - V_{1/2})/k]\}$, where V = the testing potentials, $V_{1/2}$ as the activation potential and k is the slope factor. $V_{1/2}$

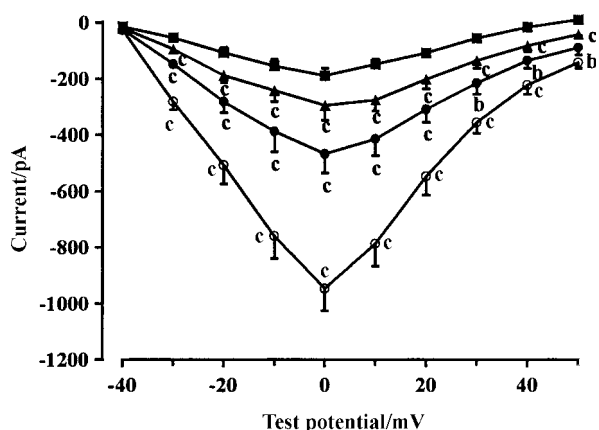


Fig 4. Effect of CPU 86017 on I - V relationship. I_{Ca} was evoked by the step pulses from 40 mV to +20 mV (HP of -40 mV). Control (O) and CPU 86017 3.3 (●), 10.0 (▲), 33.0 $\mu\text{mol/L}$ (■). $n=5-6$ cells from 4 animals. ^b $P < 0.05$, ^c $P < 0.01$ vs control.

was (-22.7 ± 4.3) mV and (-22.6 ± 4.9) mV and the k value was 6.6 ± 1.7 , and 6.0 ± 1.4 in control and CPU 86017 10 $\mu\text{mol/L}$ group, respectively ($n=6$, $P > 0.05$). The difference was not significant.

The decay of the L-type calcium current was to follow the bi-exponential equation and the time constant t_1 and t_2 of the inactivation of the maximal current at 0 mV were (88 ± 24) ms and (18 ± 6) ms in the control and (77 ± 20) ms and (15 ± 5) ms in CPU 86017 10 $\mu\text{mol/L}$ group ($n=8$, $P > 0.05$). CPU 86017 did not affect time constant of the inactivation (Fig 5).

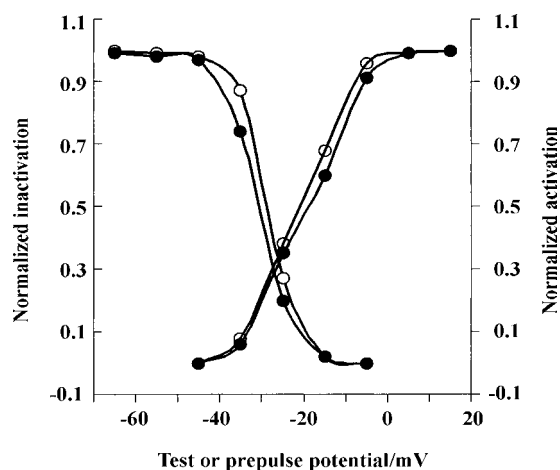


Fig 5. Effect of CPU 86017 10 $\mu\text{mol/L}$ on steady state activation and inactivation curves of I_{Ca} . Control (O) and CPU 86017 3.3 (●).

CPU 86017 inhibited contractions of rat caudal artery CPU 86017 markedly inhibited vaso-

contractions of the caudal artery induced by phenylephrine 1 $\mu\text{mol/L}$ (Fig 6, A-C) and KCl 100 mmol/L (Fig 6, D-F). The effects of CPU 86017 were stronger in phase 3 than that in phase 1 and 2 (Fig 6C and F, Tab 1).

CPU 86017 inhibited contractions induced by phenylephrine with IC_{50} of 0.465 $\mu\text{mol/L}$ in phase 1, 0.459 $\mu\text{mol/L}$ in phase 2, and 0.324 $\mu\text{mol/L}$ in phase 3. The IC_{50} of prazosin in the three phases were 0.00246, 0.00302, and 0.00173 $\mu\text{mol/L}$, respectively. The potency of CPU 86017 was 1/187 of prazosin in terms of inhibiting intracellular Ca^{2+} -induced contractions (phase 3).

CPU 86017 inhibited contractions induced by KCl 100 mmol/L with IC_{50} value of 50.2, 28.1, and 16.3 $\mu\text{mol/L}$ in phase 1, 2, and 3, respectively. The IC_{50} of verapamil was 1.16, 0.60, and 0.44 $\mu\text{mol/L}$ in phase 1, 2, and 3, respectively. The relative potency of CPU 86017 was 1/37 of verapamil.

The vasorelaxative effects of CPU 86017 on Ca^{2+} induced contractions via the α -receptor-operated Ca^{2+} channels was 50 times potent than that via voltage dependent calcium channels (Fig 6).

DISCUSSION

CPU 86017 suppressed the L-type current in isolated myocytes from guinea pigs in time- and concentration-dependent manner and did not affect the activation and inactivation curve of the current. The potency of CPU 86017 is the strongest among berberine derivatives (Tab 1). The inhibitory effects of CPU 86017 (IC_{50} 13.7 $\mu\text{mol/L}$) on L-type calcium current determined by patch-clamp are coincided with that of pacing calcium current in the sinoatrial node measured by standard microelectrode^[1], and more potent than its negative inotropic effect on atrium (IC_{50} 36 $\mu\text{mol/L}$)^[5]. In contrast, the effect of berberine on myocardial L-type calcium current is controversial. Some observed a suppressive effect^[2,3,14] and a change in inactivation curve but not activation curve^[14]. Others reported an enhancement of the inward L-type Ca^{2+} current in myocardium by berberine^[15,16] or no effect at all^[17] (Tab 1).

The vascular contractions caused by high K^{+} are attributed to the consequence of depolarization of the membrane and can be separated into three phases^[12]: 1) Phase 1 resulted from a mixed blockade on the phase

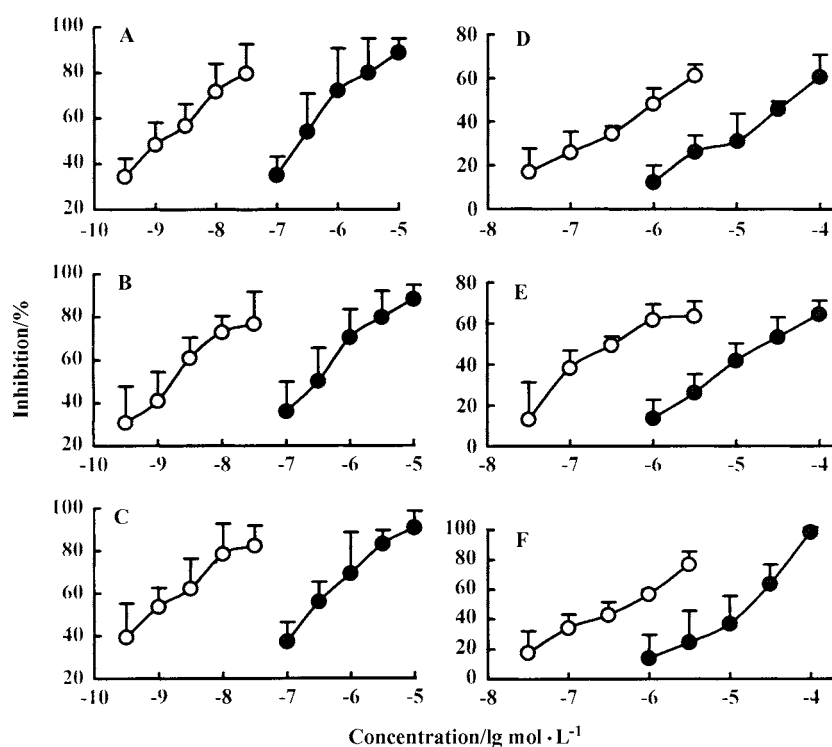


Fig 6. Inhibitory effects of CPU 86017 (●) on vaso-contraction of rat tail artery. Phenylephrine 1 $\mu\text{mol/L}$ in normal KH solution (A), Ca^{2+} free KH solution (B), and after addition of Ca^{2+} 5 mmol/L into Ca^{2+} free medium (C). KCl 100 mmol/L in normal KH solution (D), Ca^{2+} free KH solution (E), and after addition of Ca^{2+} 5 mmol/L into Ca^{2+} free medium (F). Control (○).

Tab 1. Comparison of IC₅₀ (μmol/L) of berberine and derivatives in suppression of the L-type calcium channels in myocardium.

Compounds	I _{Ca}	APD	Inotropic	Reference
CPU 86017	Suppress IC ₅₀ 11.5	Biphasic		Dai (2003)
CPU 86017	Suppressive/biphasic			Dai (1997) ^[2]
CPU 86017			Negative IC ₅₀ 36	Dai (1991) ^[5]
CPU 86035	Suppress IC ₅₀ 75			Li (2002) ^[26]
Berberine		Prolonged		Li (2001) ^[27]
Berberine	Suppressive, IC ₅₀ 30, (approximately)		Xu (1997) ^[14]	
Berberine	No effect;	Prolonged		Sanchez- (1996) ^[17]
Berberine	Enhance;	Prolonged		Wang (1997) ^[15]
Berberine	Enhance (single channel)			Zhou (1995) ^[16]
Berberine		Prolonged		Hua (1994) ^[28]
Berberine		Prolonged	Positive	Lau (2001) ^[3]
Berberine			Positive	Shaffer (1985) ^[2]
Berberine			Low concentration: positive	Chang (1952) ^[2]
Berberine			High concentration: negative	
Berberine			iv infusion positive	Maroko (1983) ^[2]
8-Oxoberberine			Positive	Chi (1997) ^[30]

2 and 3. 2) Phase 2. The released calcium provokes an amplified Ca²⁺-induced Ca²⁺ release (CICR) to implement the vasocontraction in Ca²⁺ free medium and possibly including more intracellular mechanisms^[18]. 3) Phase 3, which is developed by voltage-gated calcium channels. Phase 3 is better than phase 1 to examine Ca²⁺ channel blocking effect of a novel compound.

The α-blocking effect of berberine on aorta induced by phenylephrine was the same as that for mesenteric artery^[13,18]. The potency of CPU 86017 to suppress the contraction of anococcygeus muscle by phenylephrine^[5] is almost as same as two other derivatives of berberine to suppress aortic contractions^[19]. The potency of CPU 86017 on the caudal artery is also very closed to that on the mesenteric artery by berberine^[18]. So it can be concluded that potency of α-blocking activity of CPU 86017 was not changed. The basic structure for the α-blocking activity is rigidity and possibly related to the skeleton of berberine (Tab 2).

The blocking effect by CPU 86017 in phase 3 is significant, however, it is much weaker compared with its α blocking activity. The fact that iv injection of CPU 86017 decreased blood pressure was mainly attributed to its α-blocking effect. This is the limitation of CPU 86017 in intravenous uses. On the other hand Ca²⁺ antagonism of CPU 86017 on the vascular contractions is enhanced significantly against berberine

which exerts very weak effects to block calcium induced vascular contraction^[20]. This is in agreement with the suppressive effect of CPU 86017 on L-type calcium current in myocardium. The chemical structure of protoberberines contributing to blockade of the calcium channels is flexible, not related to the skeleton of berberine but is sensitive to both the hydrogenation and a change in side chain. In general, the derivatives of berberine show more efficacy in blocking calcium channels of myocytes and vascular smooth muscles.

The effect of berberine to prolong APD is more prominent^[2] vs CPU 86017. There are at least two determinants to control APD: $APD \propto I_{Ca}/I_K$ (I_{Kr} , I_{KS}). Usually protoberberines prolong APD by suppression of the I_K current^[2]. Berberine prolonged APD in concentration-dependent manner and an over-prolongation of APD is developed at high concentrations but it is not desired. CPU 86017 prolonged APD at low concentrations but shortened APD at higher concentration. The bi-phasic phenomena is stemmed from the two properties, reducing I_K at low concentration and blocking I_{Ca} blocking at higher concentration. The bi-phasic (or limited prolongation) pattern is an important characteristics of the novel, complex class III antiarrhythmic agents, such as Azimilide^[21] and dronedarone (SR 33589)^[22]. Complex class III agents do not induce *Torsards de pointes* (*Tdp*) very often, in contrast, the

Tab 2. Comparison of inhibitory effects of berberine and derivatives on vascular contractions by phenylephrine and high concentration of KCl.

Compounds	Artery (Rats)	α -Blockade (IC ₅₀ μ mol/L)	Calcium antagonist (IC ₅₀ μ mol/L)	References
CPU 86017	Caudal	0.324	16.3	Dai (2003)
CPU 86017	Aortic,		29.5	Dai (2000) ^[20]
CPU 86017	Aortic,		42.2	Dai (2000) ^[20]
	Aortic, diseased by <i>L</i> -thyroxin		34.3	Dai (1998) ^[12]
CPU 86017	Aortic	1.58		Dai (1999) ^[13]
CPU 86017	Aortic	2.14 (diseased by <i>L</i> -thyroxin)		Dai (1999) ^[13]
CPU 86017	Anococcygeus	pA ₂ 6.78		Dai (1991) ^[5]
Berberine	Anococcygeus	pD' ₂ 5.2		Dai (1991) ^[5]
Berberine	Mesenteric	1.48		Ko (2000) ^[18]
Berberine	Aortic		300 (approx)	Dai (2000) ^[20]
Berberine	Aortic	Potent	weak	Bova (1992) ^[31]
Berberine	Mesenteric	Potent	no effect	Chiou (1991) ^[32]
(+/-)-Govadine	Aortic	pA ₂ 6.57 (α 1-blocker)		Ko (1996) ^[19]
(-/-)-THB	Aortic	pA ₂ 6.74 (α 1-blocker)		Ko (1996) ^[19]
THB	Aortic		100 μ mol/L (47 %)	Yang (1993) ^[33]

Govadine: derivative of tetrahydroberberine; THB: tetrahydroberberine.

pure class III agents, like dofetilide^[23] and ibutilide^[24], induce *Tdp* in clinic and this limits its uses in treating ventricular arrhythmias in diseased hearts. The *Tdp* which is identical to early after depolarization will induce ventricular fibrillation. At this point CPU 86017 is superior than berberine for preventing the occurrence of *Tdp*.

Berberine possessed a positive inotropism which might be useful to treat heart failure^[25]. For a long time positive inotropism has been adopted to combat congestive heart failure but this concept has been changed in recent years after application of ACEI and new generation of β -blockers which possess dominant negative inotropism^[25]. The negative inotropism of CPU 86017 will be useful in treating congestive heart failure and pulmonary hypertension.

There are two new major merits of CPU 86017: 1) blocking the L-type channels in myocardium moderately and 2) suppressing vascular smooth muscle contraction mediated by calcium. Together with an improvement in the solubility and bioavailability, CPU 86017 will be a potential agent in new drug development.

REFERENCES

- Dai DZ, Yu F, Li HT, Tang YQ, An LF, Huang WL, *et al*.

Blockade on sodium, potassium, and calcium channels by a new antiarrhythmic agent CPU 86017. *Drug Dev Res* 1996; 39: 138-46.

- Dai DZ. The antiarrhythmic activity of protoberberines in relation to blockade of ion channels. *Ion Channel Modulators* 1997; 2: 383-90.
- Lau CW, Yao XQ, Chen ZY, Ko WH, Huang Y. Cardiovascular action of berberine. *Cardiovasc Drug Rev* 2001; 19: 234-44.
- Dai DZ, An LF, Wang YQ, Huang J, Zhang H, Dai D, *et al*. CPU 86017 suppression of arrhythmias induced by ischemia/reperfusion, ouabain, aconitine, and elevation of ventricular fibrillatory threshold. *Drug Dev Res* 1996; 39: 184-90.
- Dai DZ, An LF, Zhou MX, Zhou L, Cheng JH, Zhang JE, *et al*. Elevating effect on intramyocardial potassium level, alpha-receptor blocking effect and negative inotropism. *Bull New Drug Res Found* 1991; 1: 58-66.
- Dai DZ, He Y, Huang FH, Zhu Y, Khan HH. Comparison of inhibitory activity of berberine derivative CPU 86017 and berberine on vascular contractions. *Acta Pharmacol Sin* 2000; 31: 447-50.
- Dai DZ, Hu HJ, Yang DM, Hao XM, Zhang GQ, Zhou BA, *et al*. Chronic levo-thyroxin treatment is associated with ion channel abnormalities in cardiac and neuronal cell. *Clin Exp Pharmacol Physiol* 1999; 26: 819-21.
- Wang HL, Li SB, Dai DZ. Change of L-type calcium current in single guinea pig hypertrophic ventricular myocytes induced by levothyroxine. *J Chin Pharm Univ* 2000; 31: 130-4.
- Yang P, Lin S, Dai DZ. The effect of CPU 86017 on the RR,

- PR, QRS and QTc intervals of the ECG and blood pressure and plasma levels in infarcted dogs. *Drug Dev Res* 2002; 55: 271.
- 10 Khan HH, Dai DZ, Xiao DW, Lin S, Wang ZZ, Lou S, *et al*. Plasma CPU 86017 concentrations regarding suppression of ouabain-induced cardiac arrhythmias and decrease of heart rate in guinea pigs. *Acta Pharmacol Sin* 2000; 21: 1039-42.
- 11 Wang XL, Zhang LM, Hua Z. Blocking effect of rhynchophylline on calcium channels in isolated rat ventricular myocytes. *Acta Pharmacol Sin* 1994; 15: 115-8.
- 12 Dai DZ, Jiang JM. *p*-Chloro-benzyl-tetra-hydro-berberine inhibits vascular smooth contractions caused by Ca²⁺. *Acta Pharmacol Sin* 1998; 19: 543-7.
- 13 Jiang JM, Dai DZ, Xu SB. Effect of chlorbenzyltetrahydroberberine on normal and hyperthyroid isolated aortic rings. *Chin J Pharmacol Toxicol* 1999; 13: 131-3.
- 14 Xu SZ, Zhang Y, Ren JY, Zhou ZN. Effects of berberine on L- and T-type calcium channels in guinea pig ventricular myocytes. *Acta Pharmacol Sin* 1997; 18: 515-8.
- 15 Wang YX, Zheng YM. Ionic mechanism responsible for prolongation of cardiac action-potential duration by berberine. *J Cardiovasc Pharmacol* 1997; 30: 214-22.
- 16 Zhou Z, Lan T, Li H, Zhang Y, Wang Y. Effects of berberine on single Ca²⁺ channel current in cultured embryonic chick ventricular myocytes. *J West-China Med Univ* 1995; 26: 287-90.
- 17 Sanchez-Chapula J. Increase in action potential duration and inhibition of the delayed rectifier outward current *I_K* by berberine in cat ventricular myocytes. *Br J Pharmacol* 1996; 117: 1427-34.
- 18 Ko WH, Yao XQ, Lau CW, Law WI, Chen ZY, Kwok W, *et al*. Vasorelaxant and antiproliferative effects of berberine. *Eur J Pharmacol* 2000; 399: 187-96.
- 19 Ko FN, Chang YL, Chen CM, Teng CM. (+/-)-Govadine and (+/-)-THP two tetrahydroprotoberberine alkaloids, as selective alpha 1-adrenoceptor antagonists in vascular smooth muscle cells. *J Pharm Pharmacol* 1996; 48: 29-34.
- 20 Dai DZ, He Y, Huang FH, Zhu Y, Khan HH. Comparison of inhibitory activity of berberine derivative CPU 86017 and berberine on vascular contractions. *J Chin Pharm Univ* 2000; 31: 447-50.
- 21 Fermini B, Jurkiewicz NK, Jow B, Guinosso PJ Jr, Baskin EP, Lynch JJ Jr, *et al*. Use-dependent effects of the class III antiarrhythmic agent NE-10064 (Azimilide) on cardiac repolarization: block of delayed rectifier potassium and L-type calcium currents. *J Cardiovasc Pharmacol* 1996; 26: 259-71.
- 22 Gautier P, Guillemare E, Marion A, Bertrand JP, Tourneur Y, Nisato D. Electrophysiologic characterization of dronedarone in guinea pig ventricular cells. *J Cardiovasc Pharmacol* 2003; 41: 191-202.
- 23 Colatsky TJ. Antiarrhythmic drug: where are we going: *Pharm News* 1995; 2: 17-23.
- 24 Howard PA. Ibutilide: an antiarrhythmic agent for the treatment of atrial fibrillation and flutter. *Annals Pharmacol* 1999; 33: 38-47.
- 25 Zeng X, Zeng X. Relationship between the clinical effects of berberine on severe congestive heart failure and its concentration in plasma studied by HPLC. *Biomed Chromatogr* 1999; 13: 442-4.
- 26 Li C, Guo J, Liu T. Inhibitory action of CPU86035 on L-type calcium current in single ventricular myocyte of guinea pig. *Chin Med J* 2002; 82: 57-60.
- 27 Li BX, Yang BF, Zhou J, Xu CQ, Li YR. Inhibitory effects of berberine on IK1, IK, and HERG channels of cardiac myocytes. *Acta Pharmacol Sin* 2001; 22: 125-31.
- 28 Hua Z, Wang XL. Inhibitory effect of berberine on potassium channels in guinea pig ventricular myocytes. *Acta Pharm Sin* 1994; 29: 576-80.
- 29 Huang WM, Yan H, Jin JM, Yu C, Zhang H. Beneficial effects of berberine on hemodynamics during acute ischemic left ventricular failure in dogs. *Chin Med J* 1992; 105: 1014-9.
- 30 Chi JF, Chu SH, Lee CS, Chou NK, Su MJ. Mechanical and electrophysiological effects of 8-oxoberberine (JKL1073a) on atrial tissue. *Br J Pharmacol* 1996; 118: 503-12.
- 31 Bova S, Padrini R, Goldman WF, Berman DM, Cargnelli G. On the mechanism of vasodilating action of berberine: possible role of inositol lipid signaling system. *J Pharmacol Exp Ther* 1992; 261: 318-23.
- 32 Chiou WF, Yen MH, Chen CF. Mechanism of vasodilatory effect of berberine in rat mesenteric artery. *Eur J Pharmacol* 1991; 204: 35-40.
- 33 Yang S, Miao YS, Yan Q, Jiang MH, Jin GZ. Effects of (-)-stepholidine and tetrahydroberberine on high potassium-evoked contraction and calcium influx in rat artery. *Acta Pharmacol Sin* 1993; 14: 235-7.