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p-Chlorobenzyltetrahydroberberine inhibits vascular smooth muscle contractions caused by Ca^{2+} [1]

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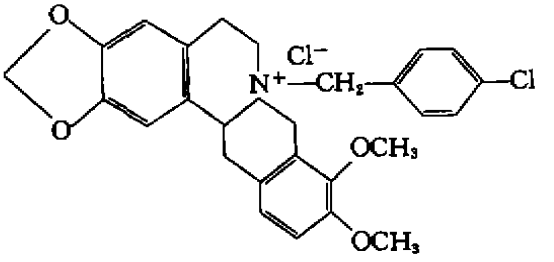
KEY WORDS calcium; vascular smooth muscle; berberine; CPU-86017; bepridil; levothyroxines; verapamil; nimodipine; thoracic aorta

AIM: To investigate influences of *p*-chlorobenzyltetrahydroberberine (CPU-86017) and levothyroxin (Lev) on vascular smooth muscle (VSM) contractions by intracellular Ca^{2+} release and calcium entry. **METHODS:** Three kinds of contractions of rat thoracic aortic rings were used to compare suppression by CPU-86017, bepridil (Bep), verapamil (Ver), and nimodipine (Nim) in euthyroid- and Lev-induced hyperthyroidism rats. **RESULTS:** The IC_{50} of CPU-86017 on KCl-induced contractions of euthyroid and hyperthyroid VSM were 80 (36 - 179) and 121 (62 - 236) $\mu mol \cdot L^{-1}$, respectively. The potency of CPU-86017 was approximate to 1/10 of Bep and 1/100 of Ver and Nim. Suppressions of Ver and Nim on hyperthyroid VSM in Ca^{2+}-free solution were greatly attenuated by -86 % and -95 %, respectively. Slight or no change in activity of CPU-86017 and Bep was found. Contractions on adding Ca^{2+} into Ca^{2+}-free medium were suppressed by CPU-86017 and its potencies in euthyroid and hyperthyroid rats were not different. **CONCLUSION:** CPU-86017 is more potent to suppress Ca^{2+} entry than intracellular calcium mobilization and Lev enhances both.

Contractions of vascular smooth muscle (VSM) by KCl in Kreb-Henseleit (K-H) solution are believed to be dependent on Ca^{2+} entry through the opening of L-type Ca^{2+} channels. Depolarization in skeletal muscle causes contractions by Ca^{2+} released from sarcoplasmic reticulum[1]. However, no contractions of VSM are caused by KCl in Ca^{2+}-free medium[2]. It is uncertain if VSM could respond to high KCl depolarization to contract in Ca^{2+}-free medium by intracellular calcium mobilization.

A blockade on voltage-dependent calcium channels in VSM by berberine was not found[2]. *p*-Chlorobenzyltetrahydroberberine (CPU-86017) possessing a marked anti-arrhythmic activity[3] shows a Ca^{2+} channels blocking effect in the heart[4]. It is interesting to find the profile of CPU-86017 on VSM contractions in comparison with classic calcium antagonists verapamil (Ver), nimodipine (Nim), and bepridil (Bep).

Levothyroxin (Lev) is active to alter VSM response to α-agonists[5]. Contractions of VSM by calcium entry and intracellular Ca^{2+} release are likely altered in hyperthyroidism in which some cardiovascular disorders develop. Therefore, we intended to explore the influence of the tested compounds in euthyroid and Lev-induced hyperthyroidism on VSM contractions to get insight of their effects on intracellular mobilization and entry of calcium in VSM.



***p*-Chlorobenzyltetrahydroberberine chloride (CPU-86017)**

MATERIALS AND METHODS

Rats Sprague-Dawley rats, weighing 200 g ± *s* 14 g and ICR mice, Grade Ⅱ of either sex used were supplied by the Animal Center of the University. (Certificate № 97004).

Chemicals CPU-86017, white crystal powder, purity > 99 % and mp 208 - 219 ℃, was kindly offered by the Center of New Drug Research and Development of the University,

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freshly prepared in K-H solution before use. Other chemicals were provided as below: bepridil (Bep) from the Changzhou Fourth Pharmaceutical Factory (lot № 930901), verapamil (Ver) from the Lianyungang Pharmaceutical Factory, nimodipine (Nim) (lot № 9303046) from the Shandong Xinhua Pharmaceutical Factory, Lev from Sigma, and norepinephrine (NE) from the Hefeng Pharmaceutical Company, Shanghai. Other reagents used were of analytical purity.

Solutions The K-H solution was freshly prepared (mmol·L^{-1}): NaCl 119, $NaHCO_3$ 25, KCl 4.6, $MgCl_2$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, and glucose 11. The Ca^{2+}-free K-H solution was prepared by adding egtazic acid 1 μmol·L^{-1} to replace $CaCl_2$.

Model of hyperthyroidism in rats[4] Rats were pretreated with Lev 1 mg·kg^{-1}·d^{-1} *po* for 8 d.

VSM contractions After equilibrium for 1 h, rat thoracic aortic rings were contracted with NE 10 μmol·L^{-1} twice to stable its activity. CPU-86017 10, 30, 100 μmol·L^{-1}, Bep 1, 10 μmol·L^{-1}, and Ver, Nim 1 μmol·L^{-1} were added to observe its suppression on 3 kinds of contractions performed as below: group 1, KCl (100 mmol·L^{-1})-induced contractions in normal K-H solution; group 2, KCl-caused contractions in Ca^{2+}-free K-H solution; group 3, contractions caused by addition of $CaCl_2$ up to 2.5 mmol·L^{-1} into the Ca^{2+}-free medium in the presence of KCl 100 mmol·L^{-1}. Suppression on the maximal contractile force ($\bar{x} \pm s$) of the normal (euthyroid) and hyperthyroid aortic rings was compared. The IC_{50} with 95 % confidence limits of CPU-86017 was calculated[6].

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared by t-test.

RESULTS

Suppressing contractions by KCl in K-H solution In euthyroid rats, CPU-86017 10 – 100 μmol·L^{-1} inhibited aortic contractions induced by KCl 100 mmol·L^{-1} in a concentration-dependent manner with IC_{50} 80 μmol·L^{-1}. (Tab 1)

Potency of CPU-86017 100 μmol·L^{-1} approached those of Bep 10 μmol·L^{-1}, Ver and Nim 1 μmol·L^{-1}, with the inhibiting rate 52 % ± 4 %, 51 % ± 8 %, 49 % ± 10 %, and 44 % ± 8 %, respectively. The activity of CPU-86017 to suppress contractions in this model was approximately 1/10 of Bep, and 1/100 of Ver and Nim. (Tab 2)

Tab 1. IC_{50} (μmol·L^{-1}) (95 % confidence limits) and potency of CPU-86017 on three kinds of VSM contractions in euthyroid and hyperthyroid rats. Row: potency to suppress contractions in euthyroid as a unity. Column: potency to suppress contractions by adding Ca^{2+} into Ca^{2+}-free medium as a unity.

Groups	Euthyroid		Hyperthyroid	
	IC_{50}	Potency	IC_{50}	Potency
Group 1	80 (36 – 179)	0.28	121 (62 – 236)	0.17
Potency	1		0.66	
Group 2	71 (34 – 150)	0.31	149 (71 – 312)	0.14
Potency	1		0.48	
Group 3	22 (13 – 37)	1	21 (11 – 40)	1
Potency	1		1.05	

Tab 2. Comparison of suppression on euthyroid and hyperthyroid rat thoracic aortic ring contractions among CPU-86017, bepridil, verapamil, and nimodipine.
$n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^fP < 0.01$ *vs* euthyroid. CF = Contractile force.

Groups/ μmol·L^{-1}	Euthyroid CF/mg	Inhibition/%	Hyperthyroid CF/mg	Inhibition/%
CPU-86017				
Control	336 ± 84		557 ± 59	
10	272 ± 76^a	19 ± 7	529 ± 55^a	4 ± 4^f
Control	499 ± 89		553 ± 52	
30	307 ± 47^c	38 ± 7	451 ± 49^c	19 ± 3^f
Control	509 ± 54		570 ± 68	
100	244 ± 17^c	52 ± 4	320 ± 36^c	44 ± 4^f
Bepridil				
Control	420 ± 51		572 ± 45	
1	358 ± 36^a	15 ± 9	442 ± 43^c	22 ± 5^d
Control	537 ± 41		545 ± 64	
10	273 ± 65^c	51 ± 8	238 ± 52^c	60 ± 9^d
Verapamil				
Control	372 ± 42		524 ± 88	
1	166 ± 31^c	49 ± 10	362 ± 74^c	32 ± 7^f
Nimodipine				
Control	477 ± 123		540 ± 65	
1	257 ± 28^c	44 ± 8	400 ± 73^c	26 ± 6^f

In hyperthyroid rats, contractions [(552 ± 17) mg, $\bar{x} \pm s$] by KCl increased up to 23 %

over the normal contractile force [(453 ± 81) mg, euthyroid], $n = 42$. $P < 0.01$. Suppression by CPU-86017 on KCl-induced contractions in this model was mildly reduced ($P < 0.01$) to half of the euthyroid, resulting in IC_{50} 121 $\mu mol \cdot L^{-1}$. Potency of Bep to suppress VSM contractions of hyperthyroid rats was not different from that of euthyroid, however, reductions of −35 % and −41 % in activity of Ver and Nim respectively, were found ($P < 0.01$) (Tab 2).

Suppression on VSM contractions caused by KCl in Ca^{2+}-free K-H solution In the normal rats strength of contractions by KCl was down to 66 %, from (453 ± 81) mg in normal K-H solution to (299 ± 76) mg in Ca^{2+}-free K-H solution. Suppression rate by CPU-86017, however, was close to those in normal K-H solution, with IC_{50} 71 $\mu mol \cdot L^{-1}$ (Tab 1). No change in potency was found with Bep 1 and 10 $\mu mol \cdot L^{-1}$, but a profound reduction in suppression by Ver and Nim was uncovered, down by −37 % and −39 %, respectively, compared with those in normal K-H solution (Tab 3).

Tab 3. Suppression by CPU-86017 on KCl 100 mmol·L^{-1}-induced contractions of rat thoracic aortic rings in Ca^{2+}-free K-H solution, compared with bepridil, verapamil, and nimodipine between the euthyroid and hyperthyroid rats. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* euthyroid. CF = Contractile force.

Groups/ $\mu mol \cdot L^{-1}$	Euthyroid CF/mg	Euthyroid Inhibition/%	Hyperthyroid CF/mg	Hyperthyroid Inhibition/%
CPU-86017				
Control	270 ± 80		330 ± 40	
10	211 ± 50[e]	22 ± 13	288 ± 32[a]	13 ± 3[d]
Control	479 ± 62		345 ± 40	
30	181 ± 49[e]	35 ± 9	267 ± 33[c]	23 ± 4[e]
Control	250 ± 56		330 ± 43	
100	110 ± 47[c]	56 ± 10	184 ± 36[c]	44 ± 10[d]
Bepridil				
Control	262 ± 55		330 ± 50	
1	228 ± 42[a]	13 ± 7	263 ± 52[b]	22 ± 6[d]
Control	292 ± 35		306 ± 45	
10	155 ± 37[c]	47 ± 11	213 ± 51[c]	35 ± 6[d]
Verapamil				
Control	259 ± 54		323 ± 50	
1	178 ± 40[c]	31 ± 10	313 ± 50[c]	5 ± 7[f]
Nimodipine				
Control	255 ± 27		308 ± 59	
1	186 ± 30[c]	27 ± 8	305 ± 58[c]	2 ± 4[f]

In Ca^{2+}-free medium contractions of hyperthyroid VSM by KCl were also enhanced by 8 %, from the euthyroid (299 ± 76) mg to (325 ± 14) mg, $n = 42$, $P < 0.05$. The contractile force was reduced to 59 % of euthyroid aortic rings [(552 ± 17) mg] in normal K-H solution, $n = 42$, $P < 0.01$, attributed to the absence of Ca^{2+} in the medium. The IC_{50} of CPU-86017 was 149 $\mu mol \cdot L^{-1}$ which was 2-fold than that in euthyroid rats (Tab 1). No difference in suppression rate of Bep 10 $\mu mol \cdot L^{-1}$ in the hyperthyroid contrasted sharply with big reductions by −84 % and −93 % in suppression by Ver and Nim, respectively (Tab 3).

Suppression on contractions on adding Ca^{2+} into Ca^{2+}-free K-H solution On adding $CaCl_2$ into Ca^{2+}-free K-H solution contractions of the euthyroid aortic rings were provoked and measured as (83 ± 12) mg. Suppression by CPU-86017 on calcium entry-induced contractions was confirmed with IC_{50} 22 $\mu mol \cdot L^{-1}$, 3 times as potent as that to suppress contractions by KCl in K-H solution, and reaching 100 % inhibition at 100 $\mu mol \cdot L^{-1}$, which was equivalent to Bep 10, and Ver and Nim 1 $\mu mol \cdot L^{-1}$ (Tab 1, 4).

Tab 4. Suppression by CPU-86017 on contractions by adding Ca^{2+} into Ca^{2+}-free K-H solution in the presence of KCl 100 mmol·L^{-1}, was compared with bepridil, verapamil, and nimodipine between euthyroid and hyperthyroid aortic rings. $n = 6$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$ *vs* euthyroid. CF = Contractile force.

Groups/ $\mu mol \cdot L^{-1}$	Euthyroid CF/mg	Euthyroid Inhibition/%	Hyperthyroid CF/mg	Hyperthyroid Inhibition/%
CPU-86017				
Control	87 ± 33		119 ± 35	
10	85 ± 25[a]	2.3 ± 3.0	95 ± 26[a]	20 ± 4
Control	101 ± 42		132 ± 44	
30	40 ± 27[b]	60 ± 10	48 ± 15[c]	60 ± 10
Control	92 ± 43		130 ± 38	
100	0[c]	100	0[c]	100[d]
Bepridil				
Control	72 ± 23		123 ± 31	
1	47 ± 25[a]	35 ± 9	63 ± 12[c]	47 ± 9
Control	88 ± 33		108 ± 30	
10	4 ± 6[c]	95 ± 7	0[c]	100[d]
Verapamil				
Control	74 ± 38		130 ± 35	
1	4 ± 4[c]	100	0[c]	100[d]
Nimodipine				
Control	68 ± 16		85 ± 14	
1	0[c]	100	13 ± 8[c]	84 ± 9[d]

In hyperthyroid rats contractions in Ca^{2+}-free medium on adding Ca^{2+} were strengthened ($P < 0.01$) to (118 ± 12) mg by 43 % increment over the euthyroid and the IC_{50} of CPU-86017 was 21 $\mu mol \cdot L^{-1}$ which was approximate to 7 times as potent as that suppressing KCl contractions of hyperthyroid VSM in K-H solution. Suppression of CPU-86017 was more potent on calcium entry contractions than those by KCl depolarization. Bep, Ver, and Nim were potent to suppress this model contractions and no change in potency was found in hyperthyroid compared with the euthyroid (Tab 4).

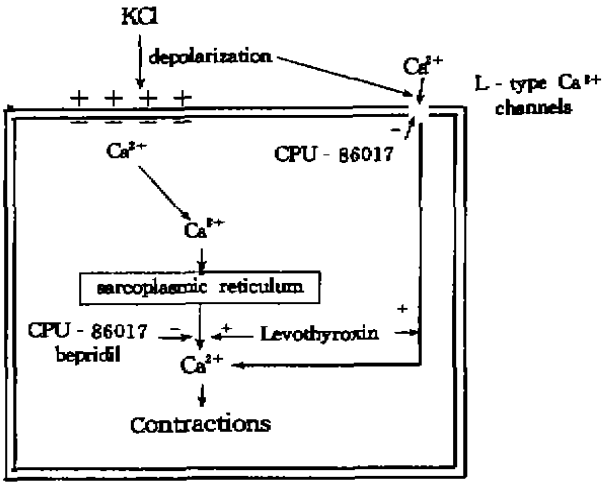

Fig 1. Two mechanisms affected by CPU-86017 and Lev, underlying VSM contractions: 1) some Ca^{2+} released from the binding to negative charges at membrane responds to KCl by depolarization, then, provoking Ca^{2+}-Ca^{2+} release mechanism which contributes to contractions in Ca^{2+}-free medium; 2) the Ca^{2+} entry via the L-type channels. CPU-86017 suppresses both but Lev enhances.

DISCUSSION

In VSM contractions caused by KCl depolarization in Ca^{2+} free medium is a matter of debate[2]. We confirm that depolarization by high KCl in Ca^{2+} free medium indeed causes contractions which are modulated by Lev, CPU-86017, and Bep.

Some Ca^{2+} binding to the negatively charged internal surface of the membrane, are likely sensitive to membrane depolarization. A release of calcium resulted while depolarization occured by high KCl provokes, in turn, a further release of calcium from sarcoplasmic reticulum via intracellular calcium mobilization[7] which is involved in KCl caused contractions in both normal and Ca^{2+} free K-H solution (Fig 1). Contractions in Ca^{2+} free KHS was totally abolished by pretreatment with thapsigargin (data not shown) known as a potent agent to deplete Ca^{2+} store by blocking Ca^{2+} ATPase[8].

Therefore, there are two mechanisms underlying contractions by high KCl depolarization: intracellular calcium mobilization and calcium entry via the L-channels. The two events are exaggerated by hyperthyroid which is more potent to enhance the first. Based upon difference in suppression on the first event Ver and Nim are classified into a same group because of less effectiveness, and Bep and CPU-86017 are more potent belonging to another group. However, CPU-86017 is potent to suppress the calcium entry[9] rather than intracellular calcium mobilization.

Bep is potent to suppress calcium intracellular mobilization and antagonize hyperthyroidism and its consequence of ventricular hypertrophy[10] and cardiomyopathy[11,12] so it is suggested, the potential, possibly together with CPU-86017, in controlling hyperthyroidism related cardiovascular disorders in clinical settings.

CPU-86017 is more potent in suppression on calcium entry than calcium mobilization, and Lev enhances both.

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对氯苄基四氢小檗碱抑制钙离子引致的血管平滑肌收缩[1]

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关键词 钙; 血管平滑肌; 小檗碱; CPU-86017; 苄普地尔; 左甲状腺素; 维拉帕米; 尼莫地平; 胸主动脉

目的: 研究 CPU-86017 及左甲状腺素(Lev)影响内钙释放及钙内流引起血管平滑肌(VSM)收缩. **方法**: 以三种大鼠胸主动脉环(VSM)收缩, 比较 CPU-86017, 苄普地尔(Bep), 维拉帕米(Ver)及尼莫地平(Nim), 在正常及 Lev 致甲亢大鼠中的抑制作用. **结果**: CPU-86017 抑制 KCl 收缩正常及甲亢大鼠 VSM 的 IC_{50}为 80 (36 – 179)及 121 (62 – 236) $\mu mol \cdot L^{-1}$, 强度为 Bep 的 1/10, Ver 及 Nim 的 1/100. Ver 及 Nim 对甲亢大鼠 VSM 在无钙 K-H 液中抑制活性大幅下降 – 86 %及 – 95 %, 而 CPU-86017 及 Bep 几乎无改变. CPU-86017 在正常及甲亢大鼠中对钙内流的抑制强度无改变. **结论**: CPU-86017 抑制大鼠 VSM 钙内流强于钙释放, 而 Lev 加强二者.