Calcium antagonistic and antiarrhythmic actions of CPU-23, a substituted tetrahydroisoquinoline

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1 The effects of CPU-23 (1-[1-[(6-methoxy)-naphth-2-yl]]-propyl-2-(1-piperidine)-acetyl-6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline) were studied on mechanical and electrical activities, and intracellular free calcium ($[Ca^{2+}]_i$) of isolated cardiac tissues in order to investigate its spectrum and mechanisms of action in the heart. Its antiarrhythmic and haemodynamic effects in pentobarbitone-anaesthetized rats subjected to coronary artery ligation were also evaluated.

2 CPU-23 at 10^{-6} – 10^{-4} M markedly inhibited slow action potential characteristics in guinea-pig papillary muscles and pace-maker action potential of rabbit sinoatrial node. It affected fast action potential only at 10^{-4} M. None of the effects of CPU-23 was reversed by washout for up to 2 h.

3 Like nifedipine and diltiazem, CPU-23 decreased the heart rate of the isolated perfused heart of the rat. However, in contrast to these two classical calcium antagonists which dose-dependently inhibited the force of contraction, CPU-23 inhibited and stimulated the force of contraction at 10^{-7} – 3×10^{-6} M and 10^{-5} M, respectively.

4 CPU-23 at 10^{-6} – 10^{-5} M inhibited the KCl-induced $[Ca^{2+}]_i$ increase in the Ca^{2+} medium, but did not affect the caffeine-induced $[Ca^{2+}]_i$ increase in the Ca^{2+} -free medium in isolated ventricular myocytes.

5 CPU-23 at 1–5 mg kg⁻¹ reduced dose-dependently ventricular arrhythmias including ventricular ectopic beats, VT and VF as well as mortality during coronary artery ligation. At 2.5–5 mg kg⁻¹ it even abolished VF, which was accompanied by 100% survival.

6 It is suggested that CPU-23 has calcium antagonistic properties in cardiac tissues. It selectively blocks the transmembrane influx of extracellular Ca^{2+} through Ca^{2+} channels, thus reducing the heart rate and developed tension, altering the slow action potential characteristics and producing antiarrhythmic effect against ischaemic arrhythmias.

Keywords: CPU-23; calcium; papillary muscle; sinoatrial node; isolated heart; arrhythmias; coronary artery ligation; heart rate; action potential; blood pressure

Introduction

Calcium antagonists are increasingly popular for treatment of various cardiovascular disorders including various cardiac arrhythmias in recent years. Their therapeutic use is based on their interference with voltage-dependent calcium channels in cardiac muscle and vascular smooth muscle (Fleckenstein, 1977; Conti *et al.*, 1985). The search for novel calcium antagonists with distinctive qualities has grown in the last few years and numerous new ones have been developed (Freedman & Waters, 1987; Ohtsuka *et al.*, 1989). With large numbers of calcium antagonists developed, several classifications have been proposed (Nayler, 1988), including one by a WHO committee (Vanhoutte & Paoletti, 1987). Many of these classifications require the use of electrophysiological or biochemical techniques to prove their ability to block specifically calcium entry into myocardial cells.

In a previous study, with a [³H]-nitrendipine binding assay we screened a series of substituted tetrahydroisoquinolines in order to develop novel calcium antagonists based on the lead compound, tetrandrine, isolated from a Chinese medicinal herb, *Stephania tetrandra* (Huang *et al.*, 1990). CPU-23 (1-[1-[(6-methoxy)-naphth-2-yl]]-propyl-2-(1-piperidine)-acetyl-6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinoline), the most potent compound identified in this series, was found to inhibit KCl-induced contraction of rat aorta and displace [³H]-nitrendipine binding in rat cerebral cortical membranes with similar potency (Dong *et al.*, 1992). It was also found

that CPU-23 behaves as a simple competitive inhibitor at the [³H]-nitrendipine binding sites (Dong *et al.*, 1992). In addition, it induces hypotension and bradycardia in rats (Dong *et al.*, 1992). It was therefore suggested that CPU-23 may exert its cardiovascular effects in inhibiting calcium influx via specific calcium channels probably L-type calcium channels (Dong *et al.*, 1992). The purpose of the present study was to characterize further the calcium antagonistic actions of CPU-23 by studying its effects on heart rate and developed tension in rat isolated hearts, action potential characteristics of guinea-pig papillary muscles and rabbit sinoatrial node, and KCl-induced increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in isolated ventricular myocytes. We also investigated antiarrhythmic and haemodynamic actions of CPU-23 in anaesthetized Sprague Dawley rats subjected to coronary artery ligation.

Methods

Langendorff perfused rat heart

Hearts were obtained from male Sprague Dawley rats weighing 200–250 g. The animals were anaesthetized with pentobarbitone (75 mg kg⁻¹, i.p.) and injected with heparin (25 u, i.p.). They were killed by cervical dislocation 10 min later. The hearts were rapidly excised and perfused through the aortic stump with a normal Krebs-Henseleit solution aerated with 95% O₂ and 5% CO₂, pH 7.4, under a constant pressure (65 mmHg) and at 34°C. The spontaneously beating preparations were allowed to adapt to the new environmental conditions for 20 min. A pressure transducer (Gould p23 ID) was connected to one opening of a three-way stopcock, the other

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two openings of which were connected to the aortic stump of the heart and the perfused solution, respectively. The heart rate and force of contraction of the isolated perfused heart were measured with a pressure transducer and recorded on a Grass polygraph (79 D). After 20 min equilibration, the test drugs were added into the perfusing solution starting from low to high concentrations. Preliminary studies showed that the effect of drugs reached a plateau at 5 min following drug administration and therefore measurement was made at 5 min following the addition of the test drug at each concentration. Each experiment was completed in about 30 min. In a preliminary experiment, it was shown that both the heart rate and force of contraction were stable during these periods in the normally perfused isolated heart.

Guinea-pig papillary muscles

Guinea-pigs (weighing 200–300 g) of either sex were anaesthetized with pentobarbitone (75 mg kg⁻¹, i.p.) and killed by a blow on the head and the hearts were quickly excised. Papillary muscles were isolated from the right ventricles, mounted in a 2 ml tissue chamber and superfused continuously at 35°C with an oxygenated (95% O₂ + 5% CO₂) Tyrode solution at pH 7.4. The preparations were electrically stimulated at 1 Hz with rectangular pulses (0.1 ms in duration, 1.5 times threshold). After the preparations had been stabilized for 1 h in normal Tyrode solution (flow rate 8–10 ml min⁻¹), the cell was penetrated by a glass microelectrode (3 M KCl, 10–20 MΩ tip resistance) and allowed to equilibrate for 30 min more. By means of conventional microelectrode techniques, action potentials were derived from stable impalements, the maximum upstroke velocity (V_{max}) was obtained by electronic differentiation and both were displayed on a storage oscilloscope (COS5020-ST) for the evaluation of action potential amplitude (APA), resting potential (RP), overshoot (OS) and the action potential duration at 50% and 90% of repolarization (APD₅₀ and APD₉₀). Slow action potentials were induced in papillary muscles as follows: after 1 h of stabilization in Tyrode solution, the preparations were superfused with a solution containing 22 mM KCl. The stimulation rate was then reduced to 0.2 Hz, the duration was increased to 1 ms and the amplitude of the stimulus intensity was quintuplicated. Addition of 10⁻⁵ M histamine induced the appearance of overshooting action potentials, the characteristics of which (low maximum diastolic potential and rate of depolarization) were typical of those of Ca-dependent action potentials (Ledda *et al.*, 1976; 1980). The following parameters were evaluated: APA, OS, V_{max} , APD₉₀ and maximum diastolic potential (MDP). After the control period, superfusion with increasing concentration of CPU-23 started and the effect of the drug was determined after 30 min superfusion with each concentration. The characteristics of both fast and slow action potentials were stable in the absence of drug for up to 2 h.

Rabbit sinoatrial node

Rabbits (1.5–2 kg) of either sex were anaesthetized with pentobarbitone (30 mg kg⁻¹, i.v.) and the hearts were quickly excised. The region of the right atrium bounded by the crista terminalis, the superior and inferior vena cava, and the interatrial septum was dissected free from the adjacent tissue in Tyrode solution at room temperature. The preparation was fixed with fine pins to the Sylgard resin (Dow Corning, Midland, MI, U.S.A.) base of a perfusion chamber (2.5 ml) and allowed to equilibrate for 1 h. The tissue was perfused with Tyrode solution at a rate 8–10 ml min⁻¹. The region between the superior and inferior vena cava was explored with microelectrodes. Sinoatrial cells were identified based on the intracellular potential profile, which shows a pronounced diastolic depolarization merging smoothly into a fast upstroke, a maximum rate of fast depolarization not greater than 10 V s⁻¹ and the small amplitude of the action potential

(Opthof *et al.*, 1985; Mugelli *et al.*, 1987). CPU-23 was administered as described in the previous section.

Measurements of $[Ca^{2+}]_i$

Ventricular myocytes were prepared from 200–250 g male Sprague-Dawley rats according to the method described previously (Young & Scarpa, 1987; Huang & Wong, 1991) with slight modifications. In brief, the heart was mounted on a modified Langendorff perfusion apparatus and perfused at 10 ml min⁻¹ with oxygenated Joklik modified Eagles medium supplemented with 1.25 mM CaCl₂ and 10 mM HEPES, pH 7.2, at 37°C for 5 min followed by 5 min in the same medium without calcium. Type I collagenase was added to this medium to a concentration of 125 U ml⁻¹ with 0.1% BSA. After 35–45 min of collagenase digestion by perfusion, both atria were discarded and ventricular tissue was dissociated by shaking in a collagenase solution for 5 min under oxygen at 37°C (pH 7.2). Following a brief trituration, the residue was filtered through 250 µm mesh screens, sedimented by centrifugation at 100 g for 1 min and resuspended in fresh Joklik solution with 2% BSA. In the cell preparation, more than 70% of the cells were rod-shaped and were not trypan-blue permeant. Calcium concentration of the Joklik solution was increased gradually to 1.25 mM in 30 min. Yields were approximately 1–2 × 10⁶ rod-shaped cells per heart in most experiments.

Myocytes were loaded with 5 µM fura 2/AM at 37°C for 40 min in Joklik solution supplemented with 1.25 mM CaCl₂, 10 mM HEPES and 2% BSA. For studying the action of the drugs on the KCl-induced increase in $[Ca^{2+}]_i$, 1–2 × 10⁴ cells were washed and resuspended in a cuvette in 2 ml normal Krebs solution with HEPES 10 mM and 2% BSA (pH 7.4). For studying the action of the drugs on the caffeine-induced intracellular Ca release, cells were washed and resuspended in 2 ml of calcium-free Krebs solution. Fluorescence measurements were carried out under constant stirring and temperature (37°C). A Hitachi F-4000 Fluorescence Spectrophotometer was used. The excitation wavelength was established with a 340 nm interference filter with a 5 nm bandpass and the emitted light was collected through a 500 nm filter with a 10 nm bandpass. The response time was 2 s. $[Ca^{2+}]_i$ was calculated according to the formula: $[Ca^{2+}]_i = 224 \times (F - F_{min}) / (F_{max} - F)$ nM (Grynkiewicz *et al.*, 1985). F_{max} was determined as the fluorescence of fura 2 after cell lysis with 33 µg ml⁻¹ digitonin while F_{min} was the fluorescence after the addition of 5 mM EGTA and 50 mM Tris. Cells were used for fluorescence measurements for up to 5 h after cell isolation with little variation in the measured parameters. Under identical conditions, comparable stimulation of unloaded cells did not produce detectable fluorescence changes.

Coronary artery ligation in anaesthetized rats

Male Sprague-Dawley rats, weighing 350–450 g were used. Animals were anaesthetized with pentobarbitone sodium (75 mg kg⁻¹, i.p.). The systemic arterial blood pressure was measured from the left common carotid artery with a Statham pressure transducer (Gould P23 ID). Heart rate was recorded with a cardiac tachometer (Model 7P4) triggered by the arterial blood pressure pulse. Arterial blood pressure and heart rate were recorded continuously with a polygraph data recording system (Grass, model 79D). The limb II lead of an electrocardiogram (ECG) was monitored continuously on an oscilloscope (Gould, Type 1425) and recorded on a Cardisun electrocardiograph (Fukuda, model 501D). A catheter was placed in a femoral vein for administration of drugs, and the trachea was cannulated to allow artificial ventilation. Rectal temperature was maintained at about 37°C. The chest was opened by left thoracotomy at the fifth intercostal space and the fifth and fourth ribs were sectioned approximately 2 mm from the left margin of the sternum. Immediately after opening the chest, the animals were ventilated with room air

Figure 1 Effects of CPU-23, nifedipine and diltiazem on the heart rate (a) and force of contraction (b) in the isolated perfused heart of the rat. Values are mean $\pm$ s.e.mean of at least 5 hearts. (○) Nifedipine; (□) diltiazem; (●) CPU-23.

using a stroke volume of approximately $2 \text{ ml } 100 \text{ g}^{-1}$ and a rate of $54 \text{ stroke min}^{-1}$. These ventilation parameters maintained arterial P_{O_2} , P_{CO_2} and pH within the normal range. After opening the pericardium the heart was exteriorised by gentle pressure on the chest wall, and a 6/0 braided silk suture attached to a 10 mm micro-point reverse cutting needle was placed under the left coronary artery as described by Selye *et al.* (1960) and Clark *et al.* (1980). The heart was replaced in the chest cavity, which was immediately closed. Any animal that exhibited arrhythmias or a sustained fall in mean arterial blood pressure (MAP) to less than 70 mmHg after the operation was discarded from further study. After an equilibration period of 15–30 min, the ligature was tied and the blood pressure and electrocardiogram were recorded for at least 30 min. When the animals died during the experiment or were killed by an overdose of pentobarbitone at the end of the experiment (4 h), the heart was removed rapidly for measurement of infarct size in order to determine if coronary ligation was successful. The hearts were rinsed under running water and the atria were discarded. The ventricles were sectioned into 3 slices from base to apex, and then stained by incubating for 15 min at 37°C in 20 ml of 0.1 M phosphate buffer, pH 7.6, containing 0.5% nitro blue tetrazolium. Infarct tissues were separated from the normal tissues, weighed, and then expressed as a percentage of the total ventricular weight. Animals with infarct size ranging from 20% to 45% were used and the infarct sizes did not differ from each other between the control and CPU-23 treated groups.

Animals were separated into 4 groups, namely, control and CPU-23-treated at 3 doses (1, 2.5 and 5 mg kg^{-1}). After a 15 min equilibration period, the animals in the control group were given saline solution and those in CPU-23-treated groups were given different doses of CPU-23 dissolved in saline solution. The saline solution was given at 1 ml kg^{-1} . The ligature was tied 15 min after saline or CPU-23 administration.

In the present study only results of early (0–30 min) post-ligation arrhythmias were presented as calcium antagonists have been shown to prevent early post-infarction ventricular arrhythmias (Fagbemi & Parratt, 1981; Curtis *et al.*, 1984; Curtis & Walker, 1986; 1988; Au *et al.*, 1987) and both mortality and arrhythmias occurred mainly during this period.

Drugs and chemicals

Nifedipine, histamine, fura 2/AM and Joklik modified Eagle's medium were purchased from Sigma Chemicals Co. (St. Louis, MO, U.S.A.). Type I collagenase was from Cooper Biomedical. CPU-23 was synthesized as previously described (Huang *et al.*, 1990) and generously provided by Professor S.X. Peng of the China Pharmaceutical University. All chemicals except nifedipine and fura 2/AM were dissolved in distilled water. Nifedipine and fura 2/AM were dissolved in dimethyl sulphoxide (DMSO). The choice of concentrations of the three calcium antagonists studied were based on previous findings in the literature and by trial and error. The highest concentrations in the experiments involving the measurement of heart rate and force of contraction in the isolated perfused heart were the concentrations that caused cardiac arrest. In the present study changes in heart rate and force of contraction in response to drugs at concentrations below the concentrations that caused cardiac arrest, were presented.

Statistical analysis

Data were expressed as mean $\pm$ standard error of the mean (s.e.mean). Statistical significance of differences between means was calculated by a paired or unpaired Student's *t* test where appropriate. A level of $P < 0.05$ was considered significant. For evaluating the differences between the incidence of arrhythmias (VT and VF) and mortality, Chi-squared test was used.

Results

Effects on the heart rate and force of contraction of the Langendorff perfused rat heart

All three drugs, nifedipine, diltiazem and CPU-23 decreased the heart rate dose-dependently, the effect of nifedipine being less obvious compared with the other two drugs (Figure 1a). Nifedipine and diltiazem markedly inhibited the force of contraction in a dose-dependent manner (Figure 1b). On the other hand, the effect of CPU-23 was biphasic, inhibiting and

Figure 2 Inhibitory effects of CPU-23 on KCl-induced increase in $[Ca^{2+}]_i$ in isolated ventricular myocytes in $1.25 \text{ nM } Ca^{2+}$ medium. Each point represents the mean of 5 separate experiments $\pm$ s.e.mean. * $P < 0.05$; ** $P < 0.01$, when compared to the control. Baseline $[Ca^{2+}]_i$ level: $128 \pm 11 \text{ nM}$. (○) Control; (●) CPU-23 $1 \mu\text{M}$; (■) CPU-23 $10 \mu\text{M}$.

Table 1 Effects of 10^{-4} M CPU-23 on fast action potential characteristics in guinea-pig papillary muscles

	RP (mV)	APA (mV)	OS (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)	V _{max} (V s ⁻¹)
Control	89.3 ± 0.9	135.5 ± 2.2	46.3 ± 2.0	151.1 ± 13.6	200.8 ± 10.4	202.5 ± 1.8
CPU-23	88.8 ± 1.0	126.0 ± 1.4*	37.3 ± 1.9*	143.8 ± 12.6*	191.3 ± 10.2*	183.5 ± 8.6*

Data shown are the mean ± s.e.mean of 4 separate experiments.

RP: resting potential; APA: amplitude of action potential; OS: overshoot; APD₅₀ and APD₉₀: action potential duration at 50 and 90% of repolarization, respectively; V_{max}: maximum upstroke velocity.

* $P < 0.05$ vs control.

Table 2 Effects of CPU-23 on slow action potential characteristics in guinea-pig papillary muscles

	MDP (mV)	APA (mV)	OS (mV)	APD ₉₀ (ms)	V _{max} (V s ⁻¹)
Control	56.2 ± 5.4	94.6 ± 4.1	33.0 ± 3.1	177.0 ± 13.2	11.0 ± 0.5
CPU-23					
10^{-6} M	54.8 ± 5.7	84.4 ± 2.9*	28.8 ± 4.3*	168.6 ± 11.8*	10.2 ± 0.6*
10^{-5} M	53.0 ± 3.0	73.0 ± 2.6*	27.7 ± 3.9*	145.0 ± 3.0*	7.3 ± 0.3**
10^{-4} M	50.7 ± 3.1	67.0 ± 6.5**	17.7 ± 3.9**	84.3 ± 4.3**	4.3 ± 0.6**

Data shown are the mean ± s.e.mean of 5 separate experiments. MDP: maximum diastolic potential.

* $P < 0.05$; ** $P < 0.01$ vs control.

Table 3 Effects of CPU-23 on maximum diastolic potential (MDP), amplitude of action potential (APA), spontaneous rate (SR) and slope of phase 4 spontaneous depolarization (dV/dt) of rabbit sinoatrial node tissue

	MDP (mV)	APA (mV)	SR (b.p.m.)	dV/dt (mV s ⁻¹)
Control	51.8 ± 3.1	38.5 ± 3.4	206 ± 10	53.0 ± 12.0
CPU-23				
10^{-7} M	52.4 ± 2.9	37.5 ± 3.1	180 ± 5*	42.0 ± 11.8*
10^{-6} M	48.0 ± 1.8	32.3 ± 4.4*	166 ± 10**	38.0 ± 10.7*
3×10^{-6} M	41.8 ± 4.0	25.0 ± 1.1*	97 ± 18**	26.8 ± 6.8*

Data shown are the mean ± s.e.mean of 5 separate experiments.

* $P < 0.05$; ** $P < 0.01$ vs control.

Table 4 Effects of intravenous CPU-23 on early post-infarction arrhythmias and mortality in pentobarbitone-anaesthetized rats

Group	n	Total number of ectopic beats	VT		VF		Mortality (% in 30 min)
			Duration (s)	Incidence (%)	Duration (s)	Incidence (%)	
Saline	10	915 ± 197	93 ± 22	100	53 ± 27	70	20
CPU-23							
1 mg kg ⁻¹	8	264 ± 130**	37 ± 26	63	2 ± 2**	13*	13
2.5 mg kg ⁻¹	6	40 ± 18**	2 ± 2**	33*	0**	0**	0**
5 mg kg ⁻¹	8	3 ± 1**	2 ± 2**	13*	0**	0**	0**

Values are means ± s.e.mean of (n) animals.

* $P < 0.05$, ** $P < 0.01$ when compared with control.

increasing the developed tension at 10^{-7} – 3×10^{-6} M and 10^{-5} M, respectively (Figure 1b). It is of interest to note that nifedipine caused cardiac arrest at concentrations higher than 3×10^{-7} M, whereas diltiazem and CPU-23 caused cardiac arrest only at concentrations higher than 3×10^{-5} M.

Effects on action potentials in guinea-pig papillary muscles

CPU-23 decreased APA and V_{max}, and shortened APD₅₀ and APD₉₀ only at a concentration of 10^{-4} M (Table 1). The effect usually occurred within 30 min after perfusion. It did not, however, affect RP and OS.

CPU-23 at 10^{-6} – 10^{-4} M dose-dependently decreased APA, OS and V_{max}, and shortened APD₉₀ of slow action potentials induced by 10^{-5} M histamine in a K⁺-depolarized guinea-pig papillary muscle without affecting MDP (Table 2).

Effects on spontaneous action potentials of rabbit sinoatrial node

As shown in Table 3, CPU-23 decreased the APA, spontaneous rate (SR) and the slope of phase 4 depolarization (dV/dt) in a dose-dependent manner. However, it did not influence MDP significantly.

Recovery of the CPU-23 effects

The effects of CPU-23 on rat isolated heart, guinea-pig papillary muscles and rabbit sinoatrial node were not reversed by superfusing with Krebs Ringer for up to 2 h.

Effects on $[Ca^{2+}]_i$ in rat ventricular myocytes

As expected, KCl dose-dependently increased $[Ca^{2+}]_i$ only in a medium with calcium, and the effect was attenuated by

Table 5 Effects of CPU-23 (given 15 min before ligation) on heart rate (HR) and mean arterial blood pressure (MAP) in anaesthetized rats subjected to coronary artery occlusion (at 0 min)

Treatment	n	HR (beats min ⁻¹)									
		-15	-10	-5	0	1	3	5	10	20	30
Saline	10	454 ± 10	450 ± 10	452 ± 10	454 ± 10	454 ± 10	455 ± 9	456 ± 9	450 ± 9	459 ± 6	459 ± 6
1 mg kg ⁻¹	8	471 ± 11	403 ± 10**	403 ± 3**	409 ± 6**	423 ± 8*	423 ± 8*	423 ± 8*	433 ± 7	435 ± 7	435 ± 8
2.5 mg kg ⁻¹	6	453 ± 12	288 ± 31**	313 ± 28**	323 ± 32*	323 ± 32*	348 ± 20**	357 ± 17**	357 ± 17**	365 ± 18**	386 ± 13**
5 mg kg ⁻¹	8	430 ± 12	243 ± 23**	276 ± 22**	283 ± 22**	311 ± 23**	318 ± 25**	319 ± 25**	351 ± 13**	363 ± 17**	373 ± 15**
Treatment	n	MAP (mmHg)									
		-15	-10	-5	0	1	3	5	10	20	30
Saline	10	104 ± 9	104 ± 9	105 ± 9	104 ± 9	71 ± 7††	87 ± 9††	92 ± 9††	92 ± 9††	93 ± 8††	94 ± 8††
1 mg kg ⁻¹	8	106 ± 7	97 ± 5	101 ± 5	103 ± 7	76 ± 4††	72 ± 4††	81 ± 9†	89 ± 10	105 ± 9	109 ± 8
2.5 mg kg ⁻¹	6	97 ± 4	71 ± 9**	78 ± 5*	79 ± 5*	70 ± 5	73 ± 4	79 ± 3	82 ± 2	86 ± 9	88 ± 8
5 mg kg ⁻¹	8	99 ± 5	53 ± 4**	69 ± 5**	76 ± 6*	66 ± 5	71 ± 6	78 ± 7	83 ± 9	86 ± 9	88 ± 8

Values are mean ± s.e. mean of (n) animals.

P* < 0.05; *P* < 0.01 vs control and ††*P* < 0.05; †††*P* < 0.01 vs 0 min.

nifedipine at 1 μM, whereas caffeine increased [Ca²⁺]_i, even in the absence of calcium (data not shown). CPU-23 at 10⁻⁶–10⁻⁵ M inhibited the KCl-induced increase in [Ca²⁺]_i in the calcium medium significantly (Figure 2), but had no significant effect on the caffeine-induced increase in [Ca²⁺]_i in the calcium-free medium (data not shown).

Effects on ventricular arrhythmias and mortality following coronary artery ligation

In agreement with the observations in a previous study (Clark *et al.*, 1980), there were mainly ventricular ectopic beats during the first 30 min post-ligation. Ventricular tachycardia (VT) and ventricular fibrillation (VF) also occurred. CPU-23 reduced dose-dependently the total number of ectopic beats, and both the duration and the incidence of VT and VF occurring in the first 30 min following coronary artery ligation (Table 4). The effects were statistically significant except for the duration and incidence of VT at 1 mg kg⁻¹ of CPU-23. CPU-23 also reduced the mortality in a dose-dependent manner. At 2.5–5 mg kg⁻¹ CPU-23 abolished completely VF, which was accompanied by 100% survival.

Effects on heart rate and mean arterial pressure following coronary artery ligation

CPU-23 dose-dependently reduced the heart rate and arterial blood pressure after its administration except at 1 mg kg⁻¹ for mean arterial pressure (Table 5). Coronary artery ligation did not affect the heart rate in saline control and CPU-23-treated rats. On the other hand, it significantly lowered the arterial blood pressure in the saline control and transiently in the rats pretreated with CPU-23 of 1 mg kg⁻¹, the lowest dose used in the study, but not in the rats pretreated with CPU-23 at doses of 2.5 and 5 mg kg⁻¹ (Table 5).

Discussion

In the present study we have further characterized the calcium antagonistic action of CPU-23 by studying the effects of the drug on (1) the heart rate and force of contraction in the isolated perfused rat heart; (2) slow action potential of the ventricular papillary muscle of guinea-pig and pace-maker potential of the sinoatrial node of rabbits, and (3) KCl-induced increase in [Ca²⁺]_i in isolated ventricular myocytes. It was found that CPU-23 dose-dependently produced effects, which are known to relate to a reduction in transsarcolemmal calcium influx. For example, the *V*_{max} of the slow action potential characteristics is an indirect index of slow inward current, *I*_{si} (Malecot & Trautwein, 1987), which may be chiefly responsible for the reduction in heart rate as observed in the present study. The reduction in force of contraction of the isolated perfused heart has been well established as resulting from inhibition of transmembrane calcium influx through the L-type calcium channels (Fleckenstein, 1977; Conti *et al.*, 1985). More importantly, CPU-23 also reduced the KCl-induced increase in [Ca²⁺]_i in isolated ventricular myocytes, providing direct evidence that the drug attenuated calcium influx. The findings render support for CPU-23 as a calcium antagonist.

Like other calcium antagonists such as nifedipine and verapamil, CPU-23 exerts antiarrhythmic action against ischaemic arrhythmias. The inhibitory effects of CPU-23 on the pace-maker action potentials in sinoatrial node, heart rate and developed tension in the rat isolated perfused heart, the slow action potential of the papillary muscle and more directly the KCl-induced increase in [Ca²⁺]_i in ventricular myocytes indicate that CPU-23 inhibits calcium influx in both pace maker and ventricular cells. In addition, CPU-23 was found to inhibit [³H]nitrendipine binding sites and the potency was correlated with its ability to inhibit KCl-induced contraction, suggesting that the drug may inhibit influx of

calcium which is required for the initiation and maintenance of VF (Merillat *et al.*, 1990) by binding to dihydropyridine-sensitive calcium channels. In short the findings of the present study support the view that the antiarrhythmic action of CPU-23 may be due primarily to an inhibition of transmembrane calcium influx in the cardiac tissue as suggested by Curtis & Walker (1988) and Lydtin & Trenkwalder (1990).

CPU-23 has other effects which may not be related to reductions in calcium influx. At 10^{-4} M, a concentration approximately 500 times the IC_{50} for inhibiting KCl-induced contraction in the rat aorta (Dong *et al.*, 1992), CPU-23 affected the fast action potential characteristics of the ventricular papillary muscle of guinea-pigs, suggesting that at this concentration, it also interferes with the sodium channels. At 10^{-5} M, a concentration that inhibits KCl-induced increase of the inward calcium current, CPU-23 increased the developed tension in the rat isolated perfused heart. Unfortunately the present study provided no information on the mechanism of the effect and further study is needed. Many calcium antagonists, including nifedipine and verapamil, depress myocardial contractility, which is harmful to patients with cardiovascular disorders as a reduction in contractility of the heart may result in heart failure (Lydtin & Trenkwalder, 1990). The fact that CPU-23 has a propensity towards stimulating myocardial contractile force at the high concentration of 10^{-5} M distinguishes it from nifedipine and diltiazem and this may be beneficial when used in treating high blood pressure in patients with cardiovascular disorders.

Like some of the other classical calcium antagonists, CPU-23 induced bradycardia and hypotension in anaesthetized rats. The decrease in heart rate is beneficial to the ischaemic myocardium as it reduces myocardial oxygen demands (Smith *et al.*, 1975). CPU-23, at the dose exerting antiarrhythmic activity, induced transient hypotension, which is also beneficial to the heart as the afterload is reduced. Interestingly, while coronary artery ligation reduced significantly the mean arterial blood pressure in the saline controls, this effect was only transient (5 min) in rats pretreated with 1 mg kg^{-1} of CPU-23, a dose that did not itself lower arterial blood pressure. Coronary artery ligation did not further reduce the mean arterial blood pressure in rats pretreated with higher doses of CPU-23 which by themselves markedly lowered arterial blood pressure. That CPU-23 did not further reduce arterial blood pressure after coronary artery ligation at doses that themselves reduced arterial blood pressure and produced significant antiarrhythmic action is beneficial to patients with cardiac arrhythmias, as arterial blood pressure would not drop further with the use of the drug during myocardial ischaemia.

The ability of CPU-23 to displace the [^3H]-nitrendipine binding in the cerebral cortical membrane suggests that it may block calcium influx by acting as a dihydropyridine antagonist at the dihydropyridine-sensitive L-type calcium channel (Dong *et al.*, 1992). Results from the previous (Dong *et al.*, 1992) and present studies showed that in addition to

the differences in efficacy in haemodynamic actions on blood pressure and heart rate, and binding affinity to the nitrendipine binding sites in the cortical cerebral membranes, there are a number of differences between CPU-23 and the prototype dihydropyridine calcium antagonist, nifedipine. First, CPU-23 causes bradycardia whereas nifedipine leads to a reflex tachycardia. Second, at a high concentration of 10^{-5} M, CPU-23 increases the myocardial contractile force while nifedipine does not have such stimulatory action. Third, the effect of CPU-23 on heart rate is far more obvious than nifedipine. These observations suggest that CPU-23 may not have the same undesirable effect(s) as nifedipine when used in treating patients with high blood pressure. Further study is needed to determine the specificity of CPU-23 as a dihydropyridine calcium antagonist and explore the possibility of its use as a therapeutic agent in the treatment of cardiovascular disorders.

In summary, in the present study CPU-23 was found to inhibit slow action potential characteristics of the guinea-pig papillary muscle and the spontaneous action potential of the rabbit sinoatrial node; to decrease the heart rate and developed tension of the rat isolated perfused heart; and inhibit the KCl-induced increased calcium influx in isolated ventricular myocytes. All these effects relate to transmembrane influx of calcium. The observation render support for the suggestion that CPU-23 may be a calcium antagonist. In addition, CPU-23 also exerts antiarrhythmic actions against arrhythmias during the first 30 min post ligation of the coronary artery in a similar way to other prototypic calcium antagonists such as nifedipine and verapamil. It is suggested that CPU-23 selectively blocks calcium influx by blocking the L-type channels and thus inhibiting activities related to calcium influx such as heart rate, developed tension in the rat isolated perfused heart, slow action potential of the guinea-pig ventricular muscle and spontaneous action potential of the rabbit sinoatrial node, and arrhythmias in the anaesthetized rat subject to coronary artery ligation. In the present study it was also found that in addition to effects related to calcium influx, CPU-23 also had other effects, which may not be related to transmembrane influx of calcium. For example, at 10^{-4} M it affected the fast action potential characteristics, suggesting it may also block sodium channels. At 10^{-5} M, a concentration that inhibits KCl-induced calcium influx, it increased the developed tension in the rat isolated perfused heart. Further study is needed to delineate the mechanism of action of CPU-23 in addition to its calcium antagonistic action.

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References

- AU, T.L.S., CURTIS, M.J. & WALKER, M.J.A. (1987). Effects of the isomers of verapamil on coronary occlusion-induced mortality and infarct size. *J. Cardiovasc. Pharmacol.*, **10**, 327–331.
- CLARK, C., FOREMAN, M.I., KANE, K.A., McDONALD, F.M. & PARRATT, J.R. (1980). Coronary artery ligation in anaesthetized rats as a method for the production of experimental dysrhythmias and the determination of infarct size. *J. Pharmacol. Methods*, **3**, 357–366.
- CONTI, C.R., PEPINE, C.J., FELDMAN, R.L. & HILL, J.A. (1985). Calcium antagonists. *Cardiology*, **72**, 297–321.
- CURTIS, M.J., MACLEOD, B.A. & WALKER, M.J.A. (1984). Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rats. *Br. J. Pharmacol.*, **83**, 373–385.
- CURTIS, M.J. & WALKER, M.L.A. (1986). The mechanism of action of the optical enantiomers of verapamil against ischaemia-induced arrhythmias in the conscious rats. *Br. J. Pharmacol.*, **89**, 137–147.
- CURTIS, M.J. & WALKER, M.L.A. (1988). The mechanism of action of calcium antagonists on arrhythmias in early myocardial ischaemia: studies with nifedipine and DHM9. *Br. J. Pharmacol.*, **94**, 1275–1286.
- DONG, H., LEE, C.M., HUANG, W.L. & PENG, S.X. (1992). Cardiovascular effects of substituted tetrahydroisoquinolines in rats. *Br. J. Pharmacol.*, **107**, 262–268.
- FAGBEMI, O. & PARRATT, J.R. (1981). Calcium antagonists prevent early post-infarction ventricular fibrillation. *Eur. J. Pharmacol.*, **75**, 179–185.
- FLECKENSTEIN, A. (1977). Specific pharmacology of calcium antagonists in myocardium, cardiac pacemakers, and vascular smooth muscle. *Annu. Rev. Pharmacol. Toxicol.*, **17**, 149–166.
- FREEDMAN, D.D. & WATERS, D.D. (1987). Second generation dihydropyridine calcium antagonists: greater vascular selectivity and some unique applications. *Drugs*, **34**, 578–598.

- GRYNKIEWICZ, G., POENIE, M. & TSIEN, R.Y. (1985). A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.*, **260**, 3440–3450.
- HUANG, W.L., SONG, X.Q., PENG, S.X. & HUANG, Z.Y. (1990). The synthesis and biological activity of substituted tetrahydroisoquinoline compounds. *Acta Pharmaceut. Sin.*, **25**, 815–823.
- HUANG, X.D. & WONG, T.M. (1991). Morphine and (D-Ala², NMe-Phe⁴, Gly-ol)-enkephalin increase the intracellular free calcium in isolated rat myocytes – effect of naloxone or pretreatment with morphine. *Life Sci.*, **48**, 1101–1107.
- LEDDA, F., MANTELLI, L. & MUGELLI, A. (1976). Blockade by burimamide of the restorative effect of histamine in tetrodotoxin-treated heart preparations. *Br. J. Pharmacol.*, **57**, 247–249.
- LEDDA, F., MANTELLI, L. & MUGELLI, A. (1980). Sympathomimetic amines and calcium-mediated action potentials in guinea-pig ventricular muscle. *Br. J. Pharmacol.*, **69**, 565–571.
- LYDTIN, H. & TRENKWALDER, P. (1990). *Calcium Antagonists: a Critical Review*. Berlin Heidelberg: Springer Verlag.
- MALECOT, C.O. & TRAUTWEIN, W. (1987). On the relationship between V_{max} of slow responses and Ca -current availability in whole-cell clamped guinea-pig heart cell. *Pflugers Arch.*, **410**, 15–22.
- MERILLAT, J.C., LAKATTA, E.G., HANO, O. & GUARNIERI, T. (1990). Role of calcium and the calcium channel in the initiation and maintenance of ventricular fibrillation. *Circ. Res.*, **67**, 1115–1123.
- MUGELLI, A., AMERINI, S., DE BONFIOLI CAVALCABO, P., CERBAI, E. & VISENTIN, S. (1987). Electrophysiological effects mediated by the stimulation of cardiac β_2 -adrenoceptors with tulobuterol. *Cardiovasc. Drugs Ther.*, **1**, 101–107.
- NAYLER, W.G. (1988). Classification of calcium antagonists. In *Calcium Antagonists*. ed Nayler, W.G. pp. 101–111. London: Academic Press.
- OHTSUKA, M., YOKOTA, M., KODAMA, I., YAMADA, K. & SHIBATA, S. (1989). New generation dihydropyridine calcium entry blockers: in search of greater selectivity for one tissue subtype. *Gen. Pharmacol.*, **20**, 539–556.
- OPTHOF, T., DE JONGE, B. & MACKAAY, J.C. (1985). Functional and morphological organization of the guinea-pig sinoatrial node compared with rabbit sinoatrial node. *J. Mol. Cell Cardiol.*, **17**, 549–564.
- SELYE, H., BAJUSZ, E., GRASSOS, S. & MENDELL, P. (1960). Simple techniques for the surgical occlusion of coronary vessels in the rat. *Angiology*, **11**, 398–407.
- SMITH, H.J., SINGH, B.N., NISBET, H.D. & NORRIS, R.M. (1975). Effects of verapamil on infarct size following experimental coronary occlusion. *Cardiovasc. Res.*, **9**, 569–578.
- VANHOUTTE, P.M. & PAOLETTI, R. (1987). The WHO classification of calcium antagonists. *Trends Pharmacol. Sci.*, **8**, 4–5.
- YOUNG, M.B.D. & SCARPA, A. (1987). Extracellular ATP induced Ca^{2+} transient in cardiac myocytes which are potentiated by norepinephrine. *FEBS Lett.*, **77**, 61–65.

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