律失常作用，且两种拮抗剂均可取消化合物48-80加重心律失常的效应，而对组胺含量无明显影响. 提示内源性组胺的致心律失常作用由 H_1 及 H_2 两种受体中介. 但目前对猫心脏组胺受体分布亚型了解较少，与大鼠心脏类似，猫心室肌组胺受体也甚稀少[9]，而 H_2 受体拮抗剂亦能对抗大鼠缺血性心律失常，提示在心肌缺血情况下心脏组胺受体可能在亲和力或数量上发生了某种变化，有待探讨.

ACKNOWLEDGMENTS 本文得到王永利讲师的协助；武占军、郭鸣放等同志协助技术工作；His测定得到本院理化仪器室王鸿志副主任技师和姜秋芬主管技师的指导.

REFERENCES

1 Wolff AA, Levi R. Histamine and cardiac arrhythmias. *Circ Res* 1986; **58** : 1
2 Li CG, Fu SX, Li YS, Xu YF. Histamine contents in hearts and whole blood during early myocardial ischemia in 3 rodents. *Acta Pharmacol Sin* 1988; **9** : 239
3 Dai S. Effects of ranitidine and cimetidine on experimentally induced ventricular arrhythmias in anesthetized rats. *Agents Actions* 1985; **17** : 460
4 Dai S. Effects of SK&F 93479 on experimentally induced ventricular arrhythmias in dogs, rats and mice. *Ibid* 1984; **15** : 131
5 Sun SY, He RR. A simple method for intracoronary injection. *Acta Acad Med Hebei* 1988; **9** : 161
6 Li YS, Fu SX, Li CG. Arrhythmogenic effects of histamine on ischemic hearts of guinea pigs. *Acta Pharmacol Sin* 1987; **8** : 536
7 Ohkura Y, Kohashi K, Tsuruta Y, Ishida S. Ultramicrodetermination of histamines by high-performance liquid chromatography. *Adv Biosci* 1982; **33** : 243
8 Dai S. Ventricular histamine concentrations and arrhythmias during acute myocardial ischemia in rats. *Agents Actions* 1987; **21** : 66
9 McNeill JH. Histamine and heart. *Can J Physiol Pharmacol* 1984; **62** : 720

中国药理学报 *Acta Pharmacologica Sinica* 1990 Nov; **11** (6) : 513-517

Effects of *m*-nisoldipine on anoxia-potentiated histamine and acetylcholine-induced contractions of the porcine isolated coronary artery

YANG Xiao-Ping, FU Shao-Xuan, LI Yun-Shan (*Department of Pharmacology, Hebei Medical College, Shijiazhuang 050017, China*)

ABSTRACT Anoxia (95% N_2 + 5% CO_2) potentiated the contractile response to KCl 20 mmol / L, histamine (His) 5 μmol / L and acetylcholine (ACh) 0.5 μmol / L in isolated porcine coronary arterial rings. Calcium antagonists *m*-nisoldipine (*m*-Nis) and nisoldipine (Nis) 0.4-250 nmol / L produced a concentration-dependent decrease in both KCl, His and anoxia-potentiated KCl, His or ACh-induced contractions. Chlorpheniramine 10 μmol / L but not cimetidine 10 μmol / L and atropine 10 μmol / L abolished contractions induced by His and ACh respectively. All 3 agents did not affect KCl response and the anoxia facilitation. Indomethacin 10 μmol / L markedly attenuated the further increase in tension by anoxia but failed to inhibit the response by these vasoconstrictors.

KEY WORDS *m*-nisoldipine; nisoldipine; indomethacin; histamine; acetylcholine; potassium chloride; coronary vessels; anoxia

Anoxia augments contractile response to KCl[1], 5-HT[1,2] and $PGF_{2\alpha}$ [3] in isolated canine coronary artery. It has been suggested that the anoxic augmentation of the contraction is relative to coronary artery spasm[2]. The mechanism of the anoxia augmentation to the vasoconstrictors is

Received 1990 Mar 12 Accepted 1990 Jun 21

uncertain. Recently an extracellular calcium dependent mechanism has been suggested, since calcium antagonist flunarizine abolished the anoxia facilitation of the contraction to $PGF_{2\alpha}$ in canine coronary artery[3]. It has also been found that anoxia facilitation of the contraction to KCl in porcine coronary artery was mediated by cyclooxygenase production[4]. The provocation of coronary artery spasm by His[5] and ACh[6] in patients with variant angina has been reported. The present studies were designed to observe 1) whether anoxia would potentiate the contraction to His or ACh in porcine coronary artery; 2) if so, whether the new calcium antagonists, *m*-Nis and His[7,8] and cyclooxygenase inhibitor, indomethacin would affect the anoxia facilitation.

MATERIALS AND METHODS

Coronary artery ring preparation The left circumflex coronary arteries were taken from freshly slaughtered porcine hearts. The middle segments of the coronary arteries were dissected and fat, adhering connective tissue were removed before rings (4–5 mm length) were cut. The rings were suspended in a water-jacketed muscle chamber filled with 20 ml of Krebs-Henseleit (K-H) solution (37± 0.5℃) and bubbled with 95% O_2 + 5% CO_2. The tension of the rings was recorded isometrically by electromechanical transducers connected to XWT-204 model potentiometric recorder. Rings were equilibrated in K-H solution for 2 h at their optimal resting tension of 3–4 g and washed every 20 min with fresh K-H solution.

Functional testing The presence of a functional endothelium was first assessed by the induction of the vessel ring contraction by the application of 5-HT (5 μmol / L). Subsequent addition of ACh 0.1 μmol / L to the bath caused relaxation. This is indicative of presence of endothelium and the release of endothelium-dependent factor[9]. Once the vessels relaxed to ACh the preparation was washed at least 3 times and left in oxygenated K-H solution for 30 min before the experiments.

Anoxia response protocol The rings were pretreated with KCl 20 mmol / L, His 5 μmol / L or ACh 0.5 μmol / L. After the contractile response had stabilized, anoxia was induced by switching from 95% O_2± 5% CO_2 to 95% N_2± 5% CO_2. Anoxia remained for 15 min. At the end of the anoxia period, oxygenation was re-established by washing with K-H solution aerated with 95% O_2± 5% CO_2. The PO_2, PCO_2 and pH values of the bathing medium were measured using a blood gas analyser (Radiometer, Copenhagen). The PO_2 values in the organ bath after 8 min equilibration period were 81.2 ± 4.3 and 5.0± 0.7 kPa for the gas mixtures of 95% O_2± 5% CO_2 and 95% N_2± 5% CO_2 respectively. The PCO_2 of the solution were 4.6± 0.5 and 4.7± 0.4 kPa. For all the gas mixtures, the pH of the solution was 7.4± 0.05.

Pharmacological antagonists pretreatment After the anoxia-potentiation contraction, the K-H solution was removed. The tissues were washed at least 3 times and left in oxygenated K-H solution for 30 min before the experiment.

Each tissue was pretreated with *m*-Nis, Nis and indomethacin for 30 min or with chlorpheniramine, cimetidine and atropine for 15 min. The anoxia challenge was replicated in the presence of the antagonist. The contraction induced by vasoconstrictor or anoxia plus vasoconstrictor in the presence of a tested agent or its solvent were expressed as developed isometric tension (g). The coronary artery rings were randomly assigned to *m*-Nis and Nis at concentrations of 0.4, 2, 10, 50, 250 nmol / L (n=5–6) and only one antagonist concentration was employed in each ring. The *m*-Nis and Nis-induced inhibition was calculated and 50% inhibitory concentration

Tab 1. Effects of vasoconstrictors (VC) or anoxia alone and anoxia-potentiated contractions to KCl, histamine (His) and acetylcholine (ACh) in porcine coronary artery rings. $\bar{x} \pm SD$. $^{***}P<0.01$ *vs* control. $^{+++}P<0.01$ *vs* VC $+O_2$. O_2 : oxygenated; N_2 ; anoxia.

Drug	*n*	Developed isometric tension (g)		
		VC+O_2	VC+N_2	N_2
Control	10	–	–	0.19 ± 0.03
KCl 20 mmol / L	6	0.76 ± 0.05	1.73 ± 0.11 $^{+++}$	0.97 ± 0.07 ***
His 5μmol / L	6	0.81 ± 0.13	1.68 ± 0.09 $^{+++}$	1.03 ± 0.08 ***
ACh 0.5μmol / L	6	1.13 ± 0.04	1.85 ± 0.07 $^{+++}$	1.11 ± 0.06 ***

(IC_{50}) and its 95% confidence limit (95% CL) were determined by regression analysis[10].

Drug preparation Indomethacin was provided by The Shijiazhuang First Pharmaceutical Factory and was dissolved in absolute alcohol. Cimetidine is the product of The Xinan Pharmaceutical Factory, it was dissolved in dilute HCl (1 mol / L) and further diluted in ion free water. *m*-Nis and Nis, obtained from Department of Organic Chemistry, Hebei Medical College, were dissolved according to our previous report[7].

Statistical analysis The data were compared using *t* test.

RESULTS

Anoxia-potentiated contraction to KCl, His and ACh Anoxia alone caused only a slight and slow increase in tension of the rings. After contractile response to KCl 20 mmol / L, His 5 μmol / L or ACh 0.5 μmol / L had stabilized, anoxia caused a further remarkable but transient contractile response which was reversible on washing with oxygenated solution (Fig 1, Tab 1). The

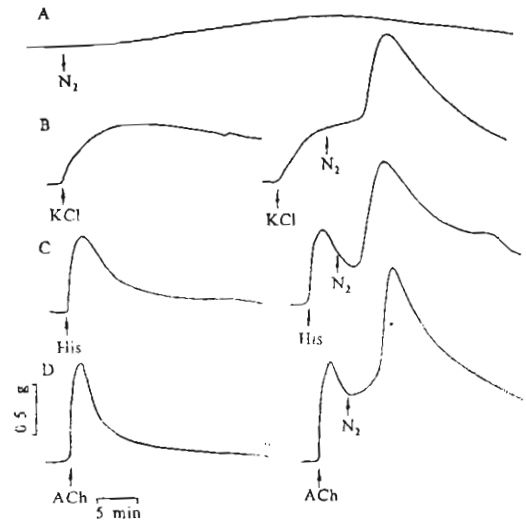

Fig 1. Anoxia-induced contraction (A) and anoxia-potentiated contraction to KCl 20 mmol / L (B), histamine (His) 5 μmol / L (C) or acetylcholine (ACh) 0.5 μmol / L (D) in isolated porcine coronary artery rings N_2: anoxia.

Tab 2. Effects of atropine, chlorpheniramine, cimetidine and indomethacin on anoxia-potentiated contractions to KCl, His and ACh in isolated porcine coronary artery rings. $\bar{x} \pm SD$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* solvent.

Drug (μmol / L)	*n*	Developed isometric tension (g)					
		KCl 20 mmol / L		His 5 μmol / L		ACh 0.5 μmol / L	
		O_2	$N_2^{\ddagger}$	O_2	$N_2^{\ddagger}$	O_2	$N_2^{\ddagger}$
Solvent	10	0.75 ± 0.06	0.95 ± 0.08	0.80 ± 0.02	1.02 ± 0.10	1.09 ± 0.08	1.08 ± 0.05
Atropine (10)	6	0.72 ± 0.04*	0.98 ± 0.09*	0.78 ± 0.06*	1.06 ± 0.09*	0.04 ± 0.03***	1.11 ± 0.08*
Chlorpheniramine (10)	6	0.71 ± 0.02*	0.93 ± 0.09*	0.01 ± 0.01***	0.96 ± 0.08*	0.93 ± 0.07*	1.02 ± 0.06*
Solvent	7	0.77 ± 0.06	0.99 ± 0.08	0.81 ± 0.07	0.97 ± 0.09	1.05 ± 0.09	1.01 ± 0.07
Cimetidine (10)	6	0.76 ± 0.09*	0.98 ± 0.05*	0.82 ± 0.03*	1.04 ± 0.12*	1.10 ± 0.07*	1.12 ± 0.08*
Solvent	6	0.73 ± 0.05	1.00 ± 0.08	0.79 ± 0.04	0.98 ± 0.09	1.05 ± 0.09	1.08 ± 0.08
Indomethacin (10)	6	0.74 ± 0.04*	0.20 ± 0.03***	0.76 ± 0.07*	0.17 ± 0.05***	1.01 ± 0.10*	0.21 ± 0.04***

‡ Net increase in tension caused by anoxia.

anoxia-potentiated conaction was repeatable over 180 min.

Effects of chlorpheniramine and atropine The contraction induced by His and ACh was nearly abolished by a low concentration (0.5 μmol / L) of chlorpheniramine or atropine, but the response to His was not inhibited by cimetidine (10 μmol / L). A relative higher concentration (10 μmol / L) of chlorpheniramine or atropine did not influence anoxia augmentation of the contraction to His or ACh and; both antagonists did not affect KCl or anoxia-potentiated KCl response (Tab 2).

Effects of *m*-Nis and Nis Calcium antagonists , *m*-Nis and Nis 0.4-250 nmol / L. produced a concentration-related depression in KCl, His-induced contraction and anoxia-potentiated contractions to KCl, His and ACh. The contraction evoked by ACh was not significantly inhibited by *m*-Nis and Nis. The difference between the IC_{50} values of *m*-Nis or Nis on KCl and His was of no significance ($P > 0.05$) (Fig 2, Tab 3).

Effects of indomethacin . Anoxia-potentiated contraction to KCl, His and ACh were all markedly attenuated by indomethacin. However, indomethacin did not affect the contractile response to KCl, His and Ach (Tab 2).

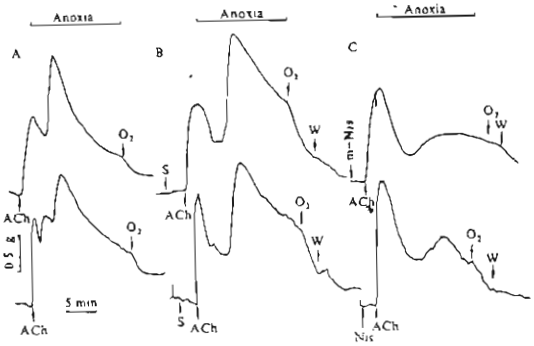

Fig 2. Effects of calcium antagonists *m*-nisoldipine (*m*-Nis) and nisoldipine (Nis) 250 nmol / L on ACh (0.5 μmol / L)-induced contraction and anoxia-potentiated contraction to ACh O_2: oxygenated; W: washing; A) control; B) solvent (S) control; C) *m*-Nis or Nis.

Tab 3. Inhibitory effects of *m*-nisoldipine and nisoldipine 0.4, 2, 10, 50, 250 nmol / L on KCl, His or ACh-induced contractions and anoxia-potentiated contraction to KCl, His or ACh in porcine coronary artery. $n = 5-6$. $^*P > 0.05$ *vs* KCl + O_2 .

		IC_{50} (nmol / L)	95% CL	*r*
			m-Nisoldipine	
KCl 20 mmol / L	O_2	4	3-6	-0.96
	N_2	12	8-18	-0.95
His 5μmol / L	O_2	8*	6-12*	-0.96
	N_2	21	16-27	-0.97
ACh 0.5μmol / L	O_2	Inhibition < 15%		
	N_2	11	9-13	-0.94
			Nisoldipine	
KCl 20 mmol / L	O_2	5	4-7	-0.96
	N_2	9	6-12	-0.95
His 5μmol / L	O_2	9*	7-11*	-0.96
	N_2	20	17-23	-0.97
ACh 0.5μmol / L	O_2	Inhibition < 15%		
	N_2	12	10-14	-0.95

IC_{50}: 50 % inhibitory concentration; 95% CL: 95% confidence limit; *r*: correlation coefficinet.

DISCUSSION

The present result that anoxia augments contractile response to KCl in isolated porcine coronary artery is consistent with previous report[4]. Anoxia has been shown to potentiate the effects of His and ACh. It has been suggested that the anoxia-potentiated contraction to 5-HT in the canine coronary artery was dependent on specific agonist receptor interaction[2]. But in our studies the anoxia facilitation did not seem to result from the specific action on H_1 or M receptor, since anoxia facilitation was obtained with KCl as well. In addition, chlorpheniramine, a H_1 receptor blocker and atropine , a M receptor blocker did not antagonized the anoxia-potentiated contraction to His or ACh, although they eliminated contractile response to His or ACh.

Our results showed that the calcium antagonists *m*-Nis and Nis effectively antagonized both the contraction evoked by KCl, His and anoxia-potentiated contractions to KCl, His and ACh. However, no significant inhibition of contraction to ACh was observed with two calcium antagonists. It is generally accepted that KCl open the "potential-operated calcium channels (POC)" and His or ACh the "receptor-operated calcium channels (ROC)". The present results indicated that there were remarkable difference of the utilizable extracellular calcium. Studies on anoxia-potentiated contraction to 5-HT[2] and $PGF_{2\alpha}$ [3] in canine coronary artery have suggested the involvement of calcium dependent mechanism. We considered the possibility that the anoxia-potentiated contraction to KCl and His as well as ACh in the porcine coronary artery was accompanied by a voltage-dependent calcium influx, since *m*-Nis and Nis remarkably inhibited the anoxia augmentation in a concentration-related manner. Our previous results[7,8] suggested that *m*-Nis and Nis showed the higher selective effect on coronary vascular bed. Therefore, *m*-Nis and Nis may be effective for relieving the coronary artery spasm.

It has been found that indomethacin, a cyclooxygenase inhibitor, inhibited anoxia-potentiated contraction to KCl[4]. Similarly, our results showed that indomethacin effectively inhibited the anoxia-potentiated contraction to His and ACh, suggesting that the production of postanoid mediate the anoxia potentiation in porcine coronary artery.

REFERENCES

1 Van Nueten JM, Van Beek J, Vanhoutte PM. Effect of Ca^{2+}-entry blockade and serotonergic antagonism on contractions of hypoxic isolated canine coronary arteries. *Arch Int Pharmacodyn* 1983; **265**: 172

2 Barrett JA, Lynch VD, Balkon J, Smith RD, Wolf PS. Effects of nitroglycerin, dipyridamole, nifedipine, verapamil and diltiazem on canine coronary arterial rings contracted with 5-hydroxytryptamine and anoxia. *Pharmacology* 1986; **33**: 139

3 Iqbal A, Vanhoutte PM. Flunarizine inhibits endothelium-dependent hypoxic facilitation in canine coronary arteries through an action on vascular smooth muscle. *Br J Pharmacol* 1988; **95**: 789

4 Rubanyi G, Paul RJ. Two distinct effects of oxygen on vascular tone in isolated porcine coronary arteries. *Circ Res* 1985; **56**: 1

5 Ginsburg R, Bristow MR, Kantrowitz N, Baim DS. Harrison DC. Histamine provocation of clinical coronary artery spasm: Implications concerning pathogenesis of variant angina pectoris. *Am Heart J* 1981; **102**: 819

6 Yasue H, Horio Y, Nakamura N, *et al*. Induction of coronary artery spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. *Circulation* 1986; **74**: 955

7 Fu SX, Li YS, Jing CJ, Ren LM. Effects of *m*-nisoldipine and nisoldipine on hemodynamics in anesthetized dogs. *Acta Pharmacol Sin* 1988; **9**: 43

8 Gao YJ, Li YS, Fu SX. Effcts of *m*-nisoldipine on the hemodynamics and regional blood flow in concious rabbits, a comparison with nisoldipine. *Chin Pharmacol Bull* 1990; **6**: 114

9 Kanamura K, Waga S, Kojima T, Fujimoto K, Itoh H. Endothelium-dependent relaxation of canine basilar arteries. Part 1: Difference between acetylcholine- and A 23187-induced relaxation and involvement of lipoxygenase metabolite(s). *Stroke* 1987; **18**: 923

10 徐端正. 回归直线的精密度. 见: 徐端正编,生物统计在药理学中的应用.北京: 科学出版社 1986: 92

间尼索地平对缺氧加强组胺和乙酰胆碱收缩离体猪冠脉的影响

杨小平、傅绍萱、李蕴山 (河北医学院药理教研室, 石家庄 050017, 中国)

提要 缺氧可加强 KCl(20 mmol / L)、组胺(His, 5μmol / L)和乙酰胆碱(ACh, 0.5 μmol / L)收缩猪冠脉环.钙拮抗剂间尼索地平(*m*-Nis)和尼索地平(Nis) 0.4～250 nmol / L 浓度依赖性地抑制 KCl、His 和缺氧加强 KCl、His 或 ACh 诱发的收缩.扑尔敏和阿托品(10 μmol / L)可分别阻断 His 或 ACh 诱发的收缩，西咪替丁(10 μmol / L)则不影响 His 的收缩，它们对 KCl 或缺氧加强缩血管物质收缩无显著影响.吲哚美辛(10 μmol / L)可显著减轻缺氧诱发的冠脉张力进一步增加，而不抑制缩血管物质的收缩反应.

关键词 间尼索地平; 尼索地平; 吲哚美辛; 组胺; 乙酰胆碱; 氯化钾; 冠状血管; 缺氧