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Effects of *m*-nisoldipine on contraction and ^{45}Ca influx in isolated rat aorta

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ABSTRACT *m*-Nisoldipine caused a concentration-dependent depression of the contractile response and ^{45}Ca influx evoked by KCl in isolated rat thoracic aorta. The IC_{50} value for contraction and ^{45}Ca influx were 0.69 (95 % confidence limits 0.32 — 1.5) nmol·L^{-1} and 0.35 (95 % confidence limits 0.06 — 2.0) nmol·L^{-1}, respectively. There was a positive correlation between the inhibition of KCl-evoked contraction and ^{45}Ca influx (r = 0.996). *m*-Nisoldipine (0.1—10 μmol·L^{-1}) did not influence the ^{45}Ca influx into resting cells and failed to inhibit noradrenaline (1.0 μmol·L^{-1})-evoked contraction and ^{45}Ca influx. The results suggest that the relaxant effect of *m*-nisodipine on rat aorta may be closely related to the blockade of Ca^{2+} entry through a potential-dependent calcium channel.

KEY WORDS *m*-nisodipine; potassium chloride; norepinephrine; calcium radioisotopes; thoracic aorta

m-Nisoldipine was first synthesized by the Department of Organic Chemistry, Hebei Medical College. We have studied its pharmacologic properties[1-3]. It shared the same characteristics with nisoldipine and was much less labile to sunlight as compared with nisoldipine. The purpose of this investigation is to provide more evidences for its inhibition of smooth muscle contractility by studying the effects of *m*-nisoldipine on ^{45}Ca influx and contractions evoked by high K^+-depolarization and noradrenaline.

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MATERIALS AND METHODS

Drugs *m*-Nisoldipine was synthesized by the Department of Organic Chemistry, Hebei Medical College. Verapamil was manufactured by Tianjing Heping Pharmaceutical Factory. $^{45}CaCl_2$ was made by Beijing Atomic Energy Institute. Egtazic acid was purchased from Sigma.

Rat Sprague-Dawley rats of either sex, 282±*s* 47 g, bred in Hebei Experimental Animal Center were used.

Tension studies Rats were killed by decapitation. Thoracic aortae were cut into spiral strips about 2 mm in width and 20 mm in length. The Krebs-Henseleit (K-H) solution contained NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 2.5, $MgSO_4$ 1.2, and glucose 11 mmol·L^{-1}, was maintained at 37 ℃ and aerated with 95 % O_2 + 5 % CO_2, pH 7.25—7.35. Aortic strips were mounted vertically in organ baths containing 20 ml K-H solution under a resting tension of 2 g. Contractile responses were recorded isometrically through a potentiometric recorder (Model: XWT-204). Each strip was allowed to equilibrate for at least 2 h, during which the incubation media were changed every 20 min. After equilibration, contraction of strip was evoked by cumulatively increasing concentrations of KCl from 10 to 80 mmol·L^{-1}. After washout and relaxation, the strips were incubated in K—H solution for 30 min, then 2 more cumulative concentration-effect curves were obtained. The maximal contractions induced by KCl 80 mmol·L^{-1} were almost the same in height. The same results were obtained in contractions induced by noradrenaline (NA) from 0.001 to 1.0 μmol·L^{-1}. The maximal response was achieved with NA 1.0 μmol·L^{-1}. After the second curve was obtained, the strips were incubated in K-H solution containing either *m*-nisoldipine or solvent for 30 min to obtain the third curve. The results were expressed as % of the agonist-induced maximal

contraction responses of 2nd curve. Control strips incubated with solvent were always rnn in parallel in each experiment. The changes in responesiveness of control strips during the whole experiment were applied to ascertain the results of the experiments.

Measurement of ^{45}Ca influx Thoracic aorta weighing about 10 mg was cnt open longitudinally. Each preparation was equilibrated in Tris buffer solution: NaCl 160, KCl 4.6, $CaCl_2$ 1.8, $MgCl_2$ 1.2, glucose 11 and Tris-hydroxymethylamino-methane 6.0 mmol·L^{-1}, gassed with 100 % O_2 at 37 C, and pH 7.4[4]. The incubation mediнm was changed every 20 min. After equilibration for 60 min, the aortic segments were exposed to ^{45}Ca (specific activity 55.5 MBq·L^{-1}) solvent or drug solntions for 5 min. Tissue ^{45}Ca nptake during this short expossnre to ^{45}Ca can be assnmed to be due to ^{45}Ca influx. The strips were pretreated with *m*-nisoldipine or verapamil for 10 min before exposure to ^{45}Ca-labeled solntion containing NA 1.0 μmol·L^{-1} or KCl 80 mmol·L^{-1} (also containing *m*-nisoldipine or verapamil), at the end of 5-min exposure to ^{45}Ca. The tissues were bathed in an ice-cold Ca^{2+}-free Tris buffer solution containing egtazic acid 2 mmol·L^{-1} for 60 min in order to remove the extracellular ^{45}Ca. Then, these strips were blotted and digested in 50 μl of a solution composed of equal parts of perchloric acid (37 % wt/vol) and H_2O_2 (30 % vol/vol). This solution was heated at 80 ℃ for 30 min. After cooled down, 5 ml of scintillation cocktail containing 0.6 % PPO and a mixture of methylacohol anhydrous and methylphenyle anhydrous were added. The radiooactivity was connted in a liquid scintillation counter (Model FJ-353). The ^{45}Ca influx was calculated by the following equation[5]:

$$^{45}\text{Ca influx } (\mu\text{mol/kg wet wt}) = \frac{\text{dpm in muscle}}{\text{wet wt (kg)}} \times \frac{\mu\text{mol of Ca/L of medium}}{\text{dpm/L of medium}}$$

Statistics Statistical significances were analyzed with *t* test. Correlation coefficent was calculated by linear regression analysis.

RESULTS

Effects of *m*-nisoldipine on KCl and NA evoked contractions *m*-Nisoldipine produced a concentration-dependent inhibition of KCl-induced contractions. The IC_{50} value was 0.69 (95 % confidence limts 0.32—1.5) nmol·L^{-1}. *m*-Nisoldipine (0.1—10 μmol·L^{-1}) showed no significant inhibition to NA-induced contractions (Tab 1). These results were consistent with those in previous reports[2,3] on rabbit thoracic aorta.

Tab 1. Effects of *m*-nisoldipine on contraction and ^{45}Ca influx induced by KCl 80 mmol·L^{-1} and NA 1.0 μmol·L^{-1} in isolated rat aorta. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

m-Nis	Contraction, mg	^{45}Ca influx, μmol/kg wet wt
	Potassium chloride	
0 nmol·L^{-1}	516±98	114±12
0.01	444±54^a	106±9^a
0.1	368±83^b	99±6^b
1.0	221±57^c	90±5^c
10.0	142±46^c	86±7^c
	Noradrenaline	
0 μmol·L^{-1}	979±128	98±10
0.1	965±135^a	97±8^a
1.0	957±117^a	96±11^a
10.0	942±108^a	94±12^a

Examination of ^{45}Ca influx method The basic ^{45}Ca influx changed according to the incubation time of preparations in ^{45}Ca-labeled Tris solution, the longer the time, the more marked the basic ^{45}Ca influx increase. The 5-min ^{45}Ca influx evoked by KCl 80 mmol·L^{-1} or NA 1.0 μmol·L^{-1} were much more than the 5-min basic ^{45}Ca influx. The values were quite different between the KCl-induced ^{45}Ca influx and the basic ^{45}Ca influx ($P<0.01$), and also between the NA-induced ^{45}Ca influx and the basic ^{45}Ca influx ($P<0.05$). Verapamil had no effect on the 5-min basic ^{45}Ca influx, but inhibited the 5-min ^{45}Ca influx evoked by KCl 80 mol·L^{-1} and NA 1.0 μmol·L^{-1} (Tab 2). The results were the same as those in a previous report[6] and indicating that the method of examining the ^{45}Ca influx was reliable.

Tab 2. Effects of different treatments on ^{45}Ca influx during 5 min in isolated rat aorta. KCl 80 mmol·L^{-1}; NA 1 μmol·L^{-1}. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* basic ^{45}Ca influx, $^dP<0.01$ *vs* KCl, $^eP<0.01$ *vs* NA.

Treatment	Verapamil (mmol·L^{-1})	n	^{45}Ca influx (μmol/kg wet wt)
Basic	0	30	76±15
Basic	0.1	6	77±10^a
KCl	0	6	118±11^b
KCl	0.1	6	60±7^d
NA	0	6	99±13^c
NA	0.1	6	71±9^e

Effects of *m*-nisoldipine on ^{45}Ca influx

Aortic strips were pretreated for 10 min in Tris buffer solution with *m*-nisoldipine before transferring to the radioactive solution. *m*-nisodipine 1 and 10 μmol·L^{-1} yielded the 5-min basic ^{45}Ca influxes, of (μmol/kg wet tussue) being 81±10 (n=6) and 75±13 (n=6), respectively, showing no significant difference *vs* controls ($P>0.05$). *m*-Nisoldipine produced a concentration-dependent inhibition on the KCl-induced 5-min ^{45}Ca influx, the IC_{50} was 0.35 (95 % confidence limits 0.06—2.0) nmol·L^{-1}. There was an excellent correlation between the inhibition of *m*-nisoldipine on KCl-induced 5-min ^{45}Ca influx and the contractions (r=0.996). The 5-min ^{45}Ca influx induced by NA was not significantly affected by *m*-nisoldipine at 0.1, 1, and 10 μmol·L^{-1}(Fig 1).

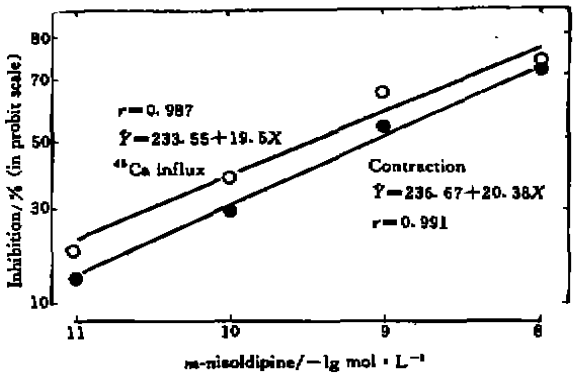

Fig 1. Inhibitory effects of *m*-nisoldipine on KCl-induced ^{45}Ca influx (○) and contraction (●) in isolated rat aorta.

DISCUSSION

The present results comfirmed that *m*-nisoldipine inhibited rat thoracic aortic strips contractions evoked by high K^+-depolarization, showing for the first time that *m*-nisoldipine blocked extracellular calcium entry evoked by membrane depolarization. This was identical with the effects of nisoldipine on contractile activtiy in isolated rabbit vascular smooth muscle[7]. The fact that the contraction of vascular smooth muscle depended on the concentration of intracellular calcium ion became evident. The high K^+-depolarization-evoked contractions were completely depended upon the influx of extracellular calcium ions through potential-dependent calcium channel. This was the reason why there existed a postive correlation between inhibitory action of *m*-nisoldipine on the KCl-induced contraction and ^{45}Ca influx. As *m*-nisoldipine did not affect the ^{45}Ca influx and contraction evoked by noradrenaline, the results showed that *m*-nisoldipine failed to block the entry of calcium ion through receptor-operated channel. This finding is at variance with that of the previous report[8] which claimed nimodipine blocked calcium ion influx through both potential-dependent channel and receptor-mediated channel. Although *m*-nisoldipine and nimodipine are both 1,4-dihydropyridine calcium antagonists, there is some minute difference between their chemical stuctures. This may be the reason why they have different effects on the contractions and ^{45}Ca influx evoked by noradrenaline in vascular smooth muscle.

In conclusion, this study indicated that the relaxant effect of *m*-nisoldipine on isolated rat thoracic aortic strips may be directly related to the blockade of the entry of calcium ions through the potential-dependent calcium channel.

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间尼索地平对大鼠主动脉收缩及^{45}Ca内流的影响

R965.2

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摘要 间尼索地平浓度依赖性地抑制 KCl 引起的大鼠主动脉收缩 $IC_{50}=0.69$ (0.32—1.5) $mmol \cdot L^{-1}$和^{45}Ca 内流 $IC_{50}=0.35$ (0.06—2.0) $nmol \cdot L^{-1}$，对两者的抑制作用有良好的相关性 ($r=0.996$)。间尼索地平0.1—10 $\mu mol \cdot L^{-1}$ 不影响静息^{45}Ca 内流，对 NA 所致的收缩和 ^{45}Ca 内流亦无影响。间尼索地平舒张大鼠主动脉的作用主要是由于阻滞电压依赖性钙通道所致。

关键词 间尼索地平；氯化钾；去甲肾上腺素；钙放射性同位素；胸主动脉

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