Effects of several Chinese crude drugs on ^{45}Ca transmembrane influx in vascular smooth muscles *

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Abstract The effects of several Chinese crude drugs including *Crocus sativus, Carthamus tinctorius and Ginkgo biloba* on Ca^{2+} transmembrane influx in rat aorta rings were studied. Resting ^{45}Ca uptake was not markedly altered by these drugs, whereas the ^{45}Ca influxes evoked by norepinephrine (1.2 μmol/L) and KCl (100 mmol/L) in rat aorta rings were significantly inhibited by *Crocus* and *Carthamus* in a concentration-dependent manner, not by *Ginkgo*. The results indicate that extracellular Ca^{2+} transmembrane influx through receptor-operated Ca^{2+} channels and potential-dependent Ca^{2+} channels can be blocked by *Crocus* and *Carthamus*.
Keywords Radionuclide ^{45}Ca, *Crocus sativus L, Carthamus tinctorius L, Ginkgo biloba L,* Rat aorta, Ca^{2+} channels

1 Introduction

The Chinese crude drugs studied in this paper including *Crocus sativus L.* and *Carthamus tinctorius L.* and *Ginkgo biloba L.* have good effects on the cardiovascular diseases. They can increase coronary blood flow and attenuate heart oxygen-consuming. However, it still remains unknown whether the mechanism is related to blocking the Ca^{2+} transmembrane influx via interacting with the cell membrane Ca^{2+} channels. Therefore, the effects of the Chinese crude drugs on Ca^{2+} influx in rat aortas are investigated by using ^{45}Ca as radiotracer.

2 Materials and methods

$^{45}CaCl_2$ was obtained from China Atomic Energy Institute with the specific radioactivity of 1.1×10^9 Bq/g Ca. The styles of *Crocus* and flowers of *Carthamus* and leaves of *Ginkgo*, which are usually used in Chinese traditional medical treatment , were adopted in the following experiments. They were extracted at 100°C with H_2O for 1 h. After filtration of the mixture, the filtrate was concentrated under reduced pressure to dryness, and dissolved in warm 0.50 volume fraction alcohol solution . Subsequent to centrifugation, the supernatant was evaporated in vacuum, and the dry residue was stored coldly and darkly.

Wistar rats (180~240 g) of both sexes were used in the experiments. The animals were stunned and sacrificed, then thoracic aortas were promptly removed and placed in physiological saline solution (PSS) containing (in mmol/L) NaCl 137 , $CaCl_2$ 1.5, $MgCl_2$ 1.0, KCl 4.6, HEPES 20, glucose 10 (pH 7.4 at 37°C). Fat and connective tissues were removed and aortas were cut into rings of about 4~5 mm long .

The procedure for the quantitation of Ca^{2+} influx in rat aorta rings was adopted from Ref.[1] with some modifications. The rings were initially equilibrated in PSS, aerated with O_2 at 37°C for 60 min . The rings were then preincubated for 3 min in $^{45}Ca^{2+}$ (3.7×10^4 Bq/ml) of PSS, followed by 5 min either in the same solution with or without norepinephrine (1.2 μmol/L) or in a K^+ -depolarizing solution of the following composition (in mmol/L): NaCl 37, $CaCl_2$ 1.5, $MgCl_2$ 1.0, KCl 100, HEPES 20, glucose 10 ($^{45}Ca^{2+}$: 3.7×10^4 Bq/ml, pH=7.4, 37°C). Incubation with extracts of Chinese crude drugs took place 40 min before the exposure to $^{45}Ca^{2+}$ and the extracts were present throughout the stimulation period. Thereafter the preparations were washed for 60 min at 0~2°C in a solu-

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tion of the following composition (in mmol/L): EGTA 10, NaCl 137, $MgCl_2$ 1.0, KCl 4.6, HEPES 10, glucose 10 (pH=7.4). Subsequently, the aorta rings were blotted dry with filter paper, weighed and dissolved in mixture of 25μl 0.70 mass fraction perchloric acid and 50μl 0.30 volume fraction H_2O_2(for each preparation) at 75°C. After cooled, scintillation solution was added, and radioactivity remaining in the tissues was detected by liquid scintillation counter using ESCR (external standard channel ratio method) as quench correction. The result of each aorta ring was converted to the apparent tissue content of Ca^{2+} (mmol per kg wet wt.) =

$$\frac{\text{Dmp in aorta}}{\text{aorta wet wt.(kg)}} \times \frac{\mu\text{mol Ca/ml solution}}{\text{Dpm/ml solution}}$$

Data are expressed as means ± standard errors. Statistical analysis was made with student's *t*-test, *P* value smaller than 0.05 was considered to be significant.

3 Results

Resting $^{45}Ca^{2+}$ uptake in rat isolated aorta was 87±9 μmol Ca^{2+} per kg wet wt. (n=10). The $^{45}Ca^{2+}$ influx was 115±13μmol Ca^{2+} per kg wet wt. (n=18) for evocation of 1.2 μmol/L norepinephrine (NE), and 143±19μmol Ca^{2+} per kg wet wt for evocation of 100 mmol/L K^+ (n=24). So the stimulants can cause the significant ascent of $^{45}Ca^{2+}$ influx as compared with resting Ca^{2+} uptake.

Table 1 Effects of Chinese crude drugs on resting ^{45}Ca uptake in rat aorta

Drugs	Concentration/ mg·L^{-1}	Ca^{2+} influx/ μmol·kg^{-1}
Control	0	87±9(n=10)
Crocus sativus L.	500	85±11(n=6)*
Carthamus tinctorius L.	500	84±10(n=6)*
Ginkgo biloba L.	400	90±12(n=6)*

*$P > 0.05$ compared with the control

Table 1 shows that resting $^{45}Ca^{2+}$ uptake was not markedly altered by *Crocus* and *Carthamus* and *Ginkgo* with respect to the control.

Table 2 shows that *Crocus* and *Carthamus* both affect the $^{45}Ca^{2+}$ influx evoked by 1.2 μmol/L NE in a concentration-dependent manner . But the influx of $^{45}Ca^{2+}$ produced by NE was not significantly influenced by *Ginkgo*.

Table 2 Effects of Chinese crude drugs on ^{45}Ca influx evoked by 1.2 μmol/L NE in rat aorta

Drugs	Concentration/ mg·L^{-1}	Ca^{2+} influx/ μmol·kg^{-1}
Control	0	115±13(n=18)
Crocus	5	108±5(n=5)*
sativus L.	50	103±6(n=5)*
	500	96±6(n=5)***
Carthamus	5	107±9(n=6)*
tinctorius L.	50	104±9(n=6)*
	500	103±9(n=6)**
Ginkgo biloba L.	400	109 ±10(n=6)*

*$P > 0.05$, **$P < 0.05$, ***$P < 0.01$ compared with the control

As illustrated in Table 3, the $^{45}Ca^{2+}$ influx evoked by 100 mmol/L K^+ was significantly blocked by *Crocus* and *Carthamus* in a concentration-dependent manner, and *Ginkgo* had no effect on $^{45}Ca^{2+}$ influx evoked by 100 mmol/L K^+.

Table 3 Effects of Chinese crude drugs on ^{45}Ca influx evoked by 100 mmol/L KCl in rat aorta

Drugs	Concentration/ mg·L^{-1}	Ca^{2+} influx/ μmol·kg^{-1}
Control	0	143±19(n=24)
Crocus	5	131±24(n=6)*
sativus L.	50	125±14(n=6)**
	500	107±6(n=5)***
Carthamus	5	137±6(n=5)*
tinctorius L.	50	126±14(n=6)**
	500	115±20(n=6)***
Ginkgo biloba L.	400	147±32(n=6)*

*$P > 0.05$, **$P < 0.05$, ***$P < 0.01$ compared with the control

The IC_{50} values were estimated from the concentration-effect correlation. The K^+ depolarization-induced $^{45}Ca^{2+}$ influx can be quantitatively inhibited by *Crocus* and *Carthamus* with IC_{50} values being 148.9 mg/L, 509.7 mg/L, respectively. And *Crocus* and *Carthamus* also inhibited the norepinephrine induced $^{45}Ca^{2+}$ influx quantitatively with IC_{50} values being 99.2 mg/L, 629.5 mg/L. It means that *Crocus* is more effective in inhibiting the $^{45}Ca^{2+}$ influx evoked by stimulants than *Carthamus*.

4 Discussion

^{45}Ca is carrier-containing radionuclide produced in nuclear reactor. So ^{45}Ca will increase Ca^{2+} concentration in solution. The increased amount can be calculated by the following formula:

$$C/40A \quad (\text{mol/L})$$

where C is radioactive concentration of ^{45}Ca in physiological solution in MBq/L; A is the specific radioactivity of ^{45}Ca in MBq/g and this value was obtained from product instruction with decay correction; the mole mass of Ca is 40 g/mol. However, the influence of this fact which could usually cause the results incorrect was not taken into account previously . In the present study , we keep the Ca^{2+} concentration in physiological solution constant by reducing the $CaCl_2$.

The ^{45}Ca influxes evoked by NE (1.2μmol/L) and KCl (100mmol/L) in rat aorta were significantly inhibited by *Crocus* and *Carthamus* in a concentration- dependent manner, whereas resting ^{45}Ca uptake was not markedly altered by these drugs. The characteristics are similar to calcium antagonists, such as verapamil. But their inhibitory effects were much less potent than verapamil, this may be due to that the effective components in extracts of Chinese crude drugs are lower. However, the extract of *Ginkgo* has no inhibitory effects on ^{45}Ca influx evoked by norepinephrine or KCl.

As reported previously,[2,3] there are two separate and distinct types of Ca^{2+} channels in smooth muscle cell membrane which regulate the entry of Ca^{2+} into cell; potential-dependent Ca^{2+} channels (PDC) would open in membrane depolarization; and receptor-operated Ca^{2+} channels (ROC) are controlled by receptor activation. The results indicate that *Crocus* and *Carthamus* both can block the potential-dependent Ca^{2+} channels and receptor-operated Ca^{2+} channels , but *Ginkgo* cannot do so. Calcium antagonism may be one of the greatest mechanisms by which *Crocus* and *Carthamus* are used for treatment of coronary heart disease. Although *Ginkgo* is efficient for cardiovascular diseases, it may have nothing to do with calcium antagonism.

References

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