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Effect of protopine on cytosolic Ca^{2+} in rabbit platelets

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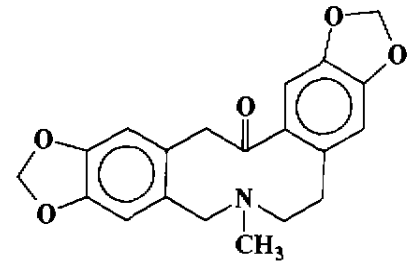
KEY WORDS protopine; adenosine diphosphate; arachidonic acids; platelet activating factor; blood platelets; calcium; verapamil

ABSTRACT

AIM: To study the influence of protopine (Pro) on the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in rabbit platelets. **METHODS**: Measurement of $[Ca^{2+}]_i$ of platelets *in vitro* by Fura 2-AM fluorescence technique. **RESULTS**: In the presence of $CaCl_2$ 1 mmol·L^{-1}, Pro 10, 20, and 40 μmol·L^{-1} attenuated the rise in $[Ca^{2+}]_i$ evoked by ADP from (420 ± 57) to (320 ± 26), (264 ± 21), and (180 ± 14) nmol·L^{-1}, respectively, by arachidonic acid (AA) from (280 ± 36) to (210 ± 17), (184 ± 21), and (143 ± 16) nmol·L^{-1}, respectively, and by platelet-activating factor (PAF) from (350 ± 42) to (282 ± 31), (223 ± 30), and (165 ± 15) nmol·L^{-1}, respectively. In the presence of egtazic acid 1 mmol·L^{-1}, Pro 10, 20, and 40 μmol·L^{-1} reduced the Ca^{2+} release induced by ADP, AA, and PAF, respectively. Pro 10, 20, and 40 μmol·L^{-1} also decreased ADP-, AA-, and PAF-induced Ca^{2+} influx. **CONCLUSION**: Pro inhibited not only Ca^{2+} release but also the influx of Ca^{2+}.

INTRODUCTION

Protopine (Pro), an isoquinoline alkaloid isolated from *Corydalis tubers*[1], showed potent inhibitory effects on platelet aggregation induced by physiologic stimuli such as ADP, arachidonic acid (AA), and Trimeresurus mucrosquamatus venom[2]. We speculated that this nonselective antiplatelet activity of Pro might be related to its influence on cytosolic Ca^{2+} level in platelets. In this study, the effects of Pro on cytoplasmic free Ca^{2+} in rabbit platelets activated by ADP, AA, and platelet-activating factor (PAF) were investigated.



Protopine

MATERIALS AND METHODS

Rabbits New Zealand white rabbits of either sex, weighing 2.0 - 3.0 kg, were obtained from Department of Animals, Yunnan Pharmacological Laboratories of Natural Products (Certificate № A12, the Administrative Commission of Medical Experimental Animals of Yunnan Public Health Bureau).

Reagents Pro (white crystalline, purity >99 %) supplied by Prof CHEN Zhi-He was dissolved in triple distilled water and stored at 4 ℃. Fura 2-AM, AA, and PAF were the products of Sigma Chemical Co. ADP was purchased from Fluka Chemical Co. All other reagents were AR and dissolved in triple distilled water.

Preparation of platelets Blood from rabbits was mixed with one-sixth volume of acid citrate dextrose (sodium citrate 85.0, citric acid 71.4, and *D*-glucose 111.1 mmol·L^{-1}). It was spun at 300 × *g* for 15 min to obtain platelet-rich plasma (PRP). PRP was further spun at 2500 × *g* for 10 min to pellet the platelets. The cells were gently suspended in warm

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Tyrode-HEPES buffer (containing NaCl 134, KCl 2.9, $NaHCO_3$ 12, NaH_2PO_4 0.36, $MgCl_2$ 1, glucose 5, HEPES 5 mmol·L^{-1}, and BSA 0.35 %, pH 7.2). Having been washed for twice, the cells were resuspended in the above buffer solution. The platelet count was adjusted to about 5×10^{11} cells·L^{-1}. Platelets were then loaded with Fura 2-AM 3 μmol·L^{-1} at 37 ℃ for 45 min. The extracellular calcium was adjusted by adding $CaCl_2$ 1 mmol·L^{-1} or egtazic acid.

Measurement of $[Ca^{2+}]_i$ Intracellular Ca^{2+} concentration of platelets was measured using Fura 2-AM (alternative excitations 340 and 380 nm, emission 500 nm) with a spectrofluorophotometer (Model RF-5000, Shimadzu, Japan) at 37 ℃ and magnetically stirred. The cells were treated with 0.1 % Triton X-100 followed by the addition of egtazic acid 10 mmol·L^{-1} to obtain the maximal and minimal fluorescences, respectively. The ratio of the measured fluorescence values at 340 and 380 nm excitation was calculated. $[Ca^{2+}]_i$ was calculated by the method of Grynkiewicz *et al*[3].

ADP (20 μmol·L^{-1})-, AA (200 μmol·L^{-1})-, or PAF (9 mmol·L^{-1})-induced rises in $[Ca^{2+}]_i$ were measured in the presence of $CaCl_2$ 1 mmol·L^{-1} or egtazic acid 1 mmol·L^{-1}.

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

RESULTS

In the presence of $CaCl_2$ 1 mmol·L^{-1}, the resting level of $[Ca^{2+}]_i$ was (98 ± 11) nmol·L^{-1} ($n = 7$); the $[Ca^{2+}]_i$ activated by ADP (20 μmol·L^{-1}), AA (200 μmol·L^{-1}), and PAF (9 mmol·L^{-1}) were (420 ± 57), (280 ± 36), and (350 ± 42) nmol·L^{-1}, respectively. In the platelets pretreated with Pro 10, 20, and 40 μmol·L^{-1}, the resting $[Ca^{2+}]_i$ were (97 ± 13), (90 ± 15), and (92 ± 10) nmol·L^{-1}, respectively, but the $[Ca^{2+}]_i$ activated by ADP (20 μmol·L^{-1}), AA (200 μmol·L^{-1}), and PAF (9 mmol·L^{-1}) were inhibited. Verapamil 80 μmol·L^{-1} decreased the above $[Ca^{2+}]_i$ elevations evoked by ADP, AA, and PAF to (251 ± 17), (171 ± 20), and (183 ± 22) nmol·L^{-1}, respectively. (Tab 1)

In the presence of egtazic acid 1 mmol·L^{-1}, the resting $[Ca^{2+}]_i$ was (73 ± 8) nmol·L^{-1}, lower than that in the presence of $CaCl_2$ 1 mmol·L^{-1}; the similar phenomenon also occurred in these 3 stimuli-activated calcium changes in platelets. Pro 10, 20, and 40 μmol·L^{-1} suppressed the $[Ca^{2+}]_i$ elevations stimulated by the 3 stimuli while not affecting the resting $[Ca^{2+}]_i$. Verapamil, however, failed to alter the resting nor activated $[Ca^{2+}]_i$ levels. Furthermore, Pro strongly inhibited the Ca^{2+} release induced by ADP, AA, or PAF. (Tab 1)

DISCUSSION

Calcium is the major intracellular regulator in platelets where there are innumerable calcium dependent activities involved in platelet shape changes, aggregation, secretion of the contents of storage granules and other processes[4]. Pro significantly inhibited ADP-, AA-, or PAF-induced $[Ca^{2+}]_i$ increase in the presence of extracellular Ca^{2+} (total rise). This demonstrated that the antiplatelet effect of

Tab 1. Effects of Pro on ADP-, AA-, and PAF-induced $[Ca^{2+}]_i$ increase in the presence of $CaCl_2$ 1 mmol·L^{-1} (total rise), Ca^{2+} release in the presence of egtazic acid 1 mmol·L^{-1}, and Ca^{2+} influx in rabbit platelets. $n = 7$ rabbits, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Drugs/μmol·L^{-1}		Total rise of $[Ca^{2+}]_i$/nmol·L^{-1}			Ca^{2+} release/nmol·L^{-1}			Ca^{2+} influx/nmol·L^{-1}		
		ADP	AA	PAF	ADP	AA	PAF	ADP	AA	PAF
Control		420 ± 57	280 ± 36	350 ± 42	115 ± 17	108 ± 25	111 ± 11	304 ± 26	174 ± 17	241 ± 21
Pro	10	320 ± 26^c	210 ± 17^c	282 ± 31^b	98 ± 12^a	99 ± 17^a	77 ± 15^c	220 ± 17^c	120 ± 14^c	201 ± 17^c
	20	264 ± 21^c	184 ± 21^c	223 ± 30^c	81 ± 13^c	77 ± 16^b	62 ± 14^c	183 ± 15^c	110 ± 11^c	164 ± 12^c
	40	180 ± 14^c	134 ± 16^c	165 ± 15^c	57 ± 11^c	61 ± 14^c	51 ± 9^c	124 ± 10^c	75 ± 9^c	109 ± 11^c
Verapamil	80	251 ± 17^c	171 ± 20^c	183 ± 22^c	107 ± 10^a	104 ± 22^a	103 ± 12^a	135 ± 16^c	70 ± 11^c	76 ± 9^c

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Pro was closely related to the inhibition of the $[Ca^{2+}]_i$ elevation. In Ca^{2+}-rich medium, the rise of $[Ca^{2+}]_i$ is largely due to an extracellular Ca^{2+} influx, and in Ca^{2+}-free (egtazic acid) medium, mainly to mobilization of intracellular Ca^{2+} stores[5]. In this experiment, the concentrations of cytosolic Ca^{2+} in resting or activated platelets were higher in the presence of $CaCl_2$ than those in the presence of egtazic acid. This suggested that the major component of the ADP, AA, or PAF-stimulated rise in Ca^{2+} was caused by the influx of Ca^{2+} ions across the plasma membrane. Pro suppressed not only Ca^{2+} influx but Ca^{2+} release as well in activation platelets, demonstrating that the antiplatelet action might be due to its inhibition of both Ca^{2+} influx of extracellular Ca^{2+} and Ca^{2+} release from Ca^{2+} store.

It is concluded that the antiplatelet effects of Pro are possibly due to the decrease of intracellular calcium concentration.

REFERENCES

1 Kin KC, Tan XC, Hsu B. Studies on the pharmacological actions. VIII. Structure-activity relationship of analogues of corydalis B (Tetrahydropalmatine). Acta Pharm Sin 1962; 9: 487 – 98.
2 Ma GY, Zhang ZZ, Chen ZH. Effect of protopine on rabbit platelet function. Acta Pharmacol Sin 1994; 15: 367 – 71.
3 Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985; 260: 3440 – 50.
4 Ardlie NG. Calcium ions, drug action, and platelet function. Pharmacol Ther 1982; 18: 249 – 70.
5 Hallam TJ, Rink TJ. Responses to adenosine diphosphate in human platelets loaded with the fluorescent calcium indicator Quin 2. J Physiol (Lond) 1985; 368: 131 – 46.

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普鲁托品对兔血小板内钙的影响

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关键词 普鲁托品; 腺苷二磷酸; 花生四烯酸类; 血小板激活因子; 血小板; 钙; 维拉帕米

目的: 研究普鲁托品(Pro)对血小板内钙离子水平的作用. **方法**: 用 Fura 2-AM 作荧光指示剂, 测定血小板内钙浓度. **结果**: 在胞外含 $CaCl_2$ 1 mmol·L^{-1}时, Pro 10, 20 和 40 μmol·L^{-1}对 ADP, 花生四烯酸(AA)和血小板激活因子(PAF)引起的血小板内钙变化分别由(420 ± 57), (280 ± 36)和(350 ± 42) nmol·L^{-1}降至(320 ± 26), (264 ± 21)和(180 ± 14) nmol·L^{-1}, (210 ± 17), (184 ± 21)和(143 ± 16) nmol·L^{-1}及(282 ± 31), (223 ± 30)和(165 ± 15) nmol·L^{-1}. 在依他酸 1 mmol·L^{-1}存在时, Pro 10, 20 和 40 μmol·L^{-1}呈浓度相关性减少 ADP, AA 和 PAF 诱导的血小板内钙释放及外钙内流. **结论**: Pro 不仅抑制血小板的内钙释放, 而且抑制其外钙内流.

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