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Effect of3 6·dimethamidodibenzOPYE don lJJn citrate on Ca2 + in rabbit platelet *in vitro'*

WEI Ba xim2 YUAN SUEFeru QIU Pei-Lun3 (*rtment of Biochemistry* [3]Department of *Pharmacology, Xi-an Medical University Xi-an 710061 China);*
YIN EPJEzu(Ihμ *ment of Pharmacology, Institute of Clinical Medical Science* Sino-Japan *Friendω osp* Bei *jing 10 9 China)'*

KEY WORDS iodonilJmαmpomds; omb ; αlcilJ1n; blcx platele ; zic acid; verapamil; lcimycin

AlM: To study e effect of 3 6- dîmethamidcr di iodonillrn citra -65) on the cyωplasIlc Qj+ <[ ]i) nmmuon in rabbit platelet. ME11IQDS: Measurement of te cyωmHcQ2+of ptatdets intpitro by using Quim 2.AM fluor lce tecm ue. RESUL1'S: m dle pn lceof CaC 1 mmol.L-1 1-65 (10 20 and 30μmol.L-1) redu d th@ rise in [Ca2 +]i induced by rombin andαlcimycin from 142 22 mnol •L-1 and 124 18nmol-L-1t0118 20 78 12 4010 Dmol.L-1 r pectively and 10815 77 14 37 ::t 14 nmol.L respc; tively. In the pr .ceof tæic acid2 mmol.L-1 1-65 (10 20 and 30 l Ibl • L-1) reduced the eaz+ :rrel indu(d by thrombin fròm 52 : 1 nmol.L-[to 34 :t9 19 ::t 6 and 11 5 nmol.L res tively. In addition 1-65 (10 20 and 30 lffi01.L-1) also reducede Ca2+ infl'1X induced by thrombin from 91 13 nmol.L-1 to 84 15 58 15 and 28 19 nmol • L res_I)E ctively. CONCLUSION: 1-65 inhibited not only the 0;+ releåse but also the influx of Ca2+ in activation platelet.

36- D immetn nidodi 1e opt qdoni cit:a (v1-65) w iodωdüa

? th i d b Departrnent of Chemistry l.. a.w*hou uruversty in 1984.The antiplatelet aggregation action of L65might be related to inhibition of thp of cytophmic freeulcium lJ In the pmem study we studied the effect of I-65on tlm* cytoplasmic free calcium ([Ca?+ IJ concentration.

MA1ERIALS AND ffiTHODS

Rωgents 1-65 synth ized by ProfαÆN shu- (ent of Chernis Lanzhou Uni)z d dissol ed in 5 % glucose solution. 2-AM was frOW WaKo Pure Chemi I dus Japan ii was obtaffied from CaffiiocMn Ine USA. Th om hi thg Bidthml Verapamil J h M de o was f10mcB j U Phmzcı.ceu c Factory. All other r e t" gents were AR and dissolved in triple dist ed water.

pre ration of platelets Bkx was J lected from the central art of rabbits pinna into plastic tubes oon16 volume of acid-citrate-d xtr (AJ and spunωobtain platelet-rich plasma *(PRP)*. The PRP was acidi pH 6.6 with ci ic acid 0.15 mol.L and spun (900×*g* for 10 rnin). The platelet peliet was suspended in buffer A ωntaining NaCl 145 KCl 5 Mg 1 NaI 14 0.5 HEPES 10 dextrose 2 tazic acicl. 2 mmol.L-1 and BSA 2.3 %HH 6.5. 1t was pun again at 900×g for 10 min and r uspended at 2 x 10^{11} platelets.L-1 in buffer B ntaining NaCl 145 KCl 5]\.1g$C4 1 N 0.5 E PES 10 dextrc 2 mmol.L-1 pH 7.4-.

Meas EE ent of cytωωolic 0;+ In acellular C2+ :Z ! on of d was m d usingQuin 2

hlu'mbinduced rise in [ea2+ 1 was ffil nro separately in the pr ence of $CaCl_2$ 1 mmol.L-1 and tmc acid 2 mmol.L 1.Fα. the latter Quin 2-AM..læded plaωlet suspensions were incubated Wtb 5 19 at 3rt for 5 min. with egtazic acid 2 nunol.L-1 beføre activation. The me m[Ca2+]i in presence of CaCl21mmol-L-lmpmmsa cαnbination of Ca2+ release and influx of ea2+. The rise in the pr ence of tazic acid 2 mmol.L-1 reflec ea2+ release. The difference between the 2 measurer dω of XtmlaiCab.

atistics All data were *edasx:!:S.* *p* value was evah ated by *t* t t.

RESUEI3

In the presence of Cach 1 mmol.L-1 in rabbit

;22 35 *ZZmNa Natural* Scíen Oanda on of
2ωη pondence to Th:- *WEI* &0-. a. n*now Nω* nal Cen*lω*. før Cli al La *mWTY:1 Eh h R d ijt g* 100730Ch ina
, 9602 5 Ac *td1996·1233*

platelets, e mdrIg[02+]i was67±12'mol 8). Thrombin 50 u_. L-and JA 200nEnol-fsm a rise in calcimycin from the resting level to 210 32 ([d+;:1H d 191 18 (P<0.01 n = 8) -!:;wy'III M-m dets nt 19 120 and 30 no1·L-vthe resttrtg I-65 10, as 69 10 64 15 and 63 :t 12 nol [Ca2+]i :8) respectively indicating the resting ·L-1 (n 2+1. was maffectd; but I-65 markedly ;Jidωtal rise in [ ]i induced by thrombin afld caicimycin (Tab 1)·

im t of 1-65ωrimM[CaF]ih ced by and ca1 e pn of C 2 1 mmol TT(ωωrise) on ea2+ rell d by nbin in Ld tsic a 'ωmd| L- MK rabbit pla lets. n c:: 8 rabbits s. ? 0.05 ?<0.05 ?<0.olv?ω . [.

[Ca4+ 1/nmol.L-1

Bug/Tot4dseQUInflux

101.L-1		Thrombin-induced		Calcirnycin-induced		release			
}..65	0	142:t 22		124	18	52	11	911:13	
	10	118	20[a]	108	15[a]	341:9[b]		84	15[a]
	20	781:12[c]		ii	:t13[c]	191:6[c]		58	15[c]
	30	40	10[c]	31	14[c]	111:5[c]		481:9[c]	
verapamil	100	63	11[c]	541:t2[c]		48	10[8]	16	9c

ηle rise in [C;+]i over resting levels induced by ombin in the presence of egtazic acid 2 mmol ·L-1 w Snot diminished by verapamil but was diminished by 1-65 (Tab 1).

I-65 markedly inhibited the influx of 01+ induced by throm:bin (influx was calculated as the differ ce in the rise in '[Ci Ji noted when platelets were activated in the presence ofCaC12 1 mrnol'L-1 and egtazic acid 2 mmol.L-1) (Tab 1).

DISCUSSION

An rly platelet resIX>nse to activation is a rise cytoplasmic [ea2+ Ji which plays 'an important tole m stimulus-response coupling 44 - riment demonstrated at 1-65 markedly i bited the rise in [Ca2+]i indmced by calcimycin Ironlbin in pmmce extracellular Ca2+(totd n This suggested that the antiplatelet action of 1-65. was closely related to the inhibition of the rise in [Ca2+]i·

In this-study the effects of 1-65 on .e ea2+ r@lease and influx of 0l-+ induced by thrombin were obs ved separately. The results showed that 1-65 inhibited not oruy the Ca2+ release but also the inflw of Ca2+ in activation platelet. Since the agonist-induced rise in [Ca2+ Ji in platelet is a m bination of Ca2+ release from Ca2+ store and influx of extracellular ea2+ (7) these findings lead to the nclusion that the principle of antiplatelet of 1-65 is mainly due to the inhibitian in both Ca2+ release and influx of Ca2+.

Because myocardial cell in intake storage and release of Ca2+ is s 1 to platelet. Therefore le-ant -CaZ+ mobilirzation action in platelet of 1...65 may help to elucidate its protective effects on my dial injucy by ischemia and reperfusion(8).

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3,6-(二甲氨基)-二苯骈碘杂六环枸橼酸盐体外对

2. (:
ija) : (tl
.ft }
·L⁻¹)使血小板[Ca²⁺]ᵢ分别由 142 22 nmol·L⁻¹
124 18 ronol-L-1 1l8 78 12..
10 nnxl" L-1 1 1'5 14 37 14
; 1 : Z 2 Om L-1 a
1-65 CiJ. 52 11 mn . L'-1
L 霉素 减少
: i65 91 13 L-iF
<(Ca²⁺ 1) - : Qllîn Alvl 降至 84 15 58 5 28 19 runot. L-1 别
[C...+]i. t ±65 ! Ca. It 结
C 2 1 f.llIn)!L I 1 5 (l() 20 30μrool CaZ . 颗

j]lIJD; :\02 -9i56 Atn Pl m'18cd m smca 1997 Juh 18 (4): 358-362

Inhibitory effects of copper-aspirin cømplex on platelet aggregatìon[1]

S N Zhì-Qiang. CHE Zhi-He W1A Guo-Yi WAl G EKFCbmg WU Wan-LingUhmn
*Pha.rmacoiogi*ωI Laboratorirs ofNaturai prducfs Kunming MedtCGL Cbtiegekt4717 *i'1g 65*ω3J' *chi*
L1lJ Wei-Ping \]G Yi-KunXIONG Hui- Zhou
(fcumiJIg IFLEdf qfpredoω *Metals Kunrni 6J0221China)*

KE'y' " ORDS P platelet agglega- tm; rornbaxane A_2 ;)Ɵɪ i oi;motomII; douic acid; adenc diphc phate; f!latelet activa !actor

All\f:To study the înbïbitory effocts of PF asp α Ile (u sp) on platelet a ation. ME'!BODS: wi ad10Sine diphosphate the effects of tuAsp platelet ag egation vitro or *in vivo* wmmv aud. RaEHokmm ssay Wfluo:r:0 plx e were eà measure thrombox.ane '.l (1'Xl) neratioll froro platelets e levels of T and of 6-ket -PGFlαin plSSIlla and the platelet >ninrelæse reacti RFSULTS: *In* l.I*tro* CuAsp inlited arachidoIÚç acid (AA)-induced gregãtion (ICso =17 unol.L-1 95 % nfid 1.Ce limits: 9 – 33μmol.L-[1])e rel se of 5-Hf (I(: : :19μmol•L--1 95 % nfidence limits: 10 – 30 101 • L-1) andτχgeneration from p1a (*P*< 0.05). Usp 10 ¹ mg .. —› 1ig tively inhibíted M.-induced g pb: increased e 6-keto- F1a t1Centration in m while decreased at of rrx . ωNÇL{ : CuAsp *in vi* shows lle iuhil .tory effects on AA-indu d a D. tt. i i (Asp) r lated to inhibition of p1a cyclooxygenase and. e release of active m platelets.

Aspirin (Asp) shows a lt. telel aggregation effect and is vvidely appHed to the prevention and therapy of thron1OC noolic especially ischemic c diovωar di ases(l). A at higher dæes however supress pros a (Pro) synthesisn endothelial cells while i nhibits thromooxane (TXA_2) gen ration in pl platelets. To look for a substitute of Asp has be mea Qf interest.

Copper aspirinate [O aspirinate)4 CuAsp], a pper mplex Qf Asp(had t10t Iiy more potent ar ti-inflammatory, and antt4a 1tMl ie elfeßts(2,3) but fewer gast:0αintt stinal side eÆfects than Asp' due to the

1t S11pported by e Fund for the Study df Basic Usage of Y1.1ma.an ProvinceN94ωω2r
Rec ved 1996-02-12 A eptoo 1997-02-19