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()f berbeJ'ine on L- and T..t : V cul mm"zpe i\1111 ch els

XIJShang-mong2 2 NG ye REN Ji YZHOU Zhao Nì

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UY WORDS M dinez I " " ClaUlp chniques;

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Wb;ddmcMmh

IM! To sωdytlts of rine' (Ber) on L ZJL)adT T) cbannels in 001 gum niDVen cularmyl e8. ME ODS: Using whole LAm chznpmdbnique. RESULTS: 30pmol.L-[1] Iω L 'o Iω 241 PAω978 pA (*n*=5 cellsof5 ea pip P b.05) adω" 179 pA (*n*=5 P<O.Ol) respectively the ibitorv effect was c .cen — dependent and non- ua . t. The ak vahe of 1CaL α volå 'e relati p was decreased Ber atIeC M6v m netics of / . 1be baJf vation po 1 (Vo.s) was from -27.8mVω 34.2 mV and βslope :(κ)wωcban 9.22 into 13.03.. B did 1K d vation m6ω. Ber 10 and 30 001- L-1 aISOinJul d Jea m154 pAω 101 78 pA and ω48 45 pA *n* = 8 cells of 5 m gs *P* < 0.05). CONCLUSION: Ber Pl K.... bkx D2 effec on th L- and T BI 1chanr —

Berberine (Ber) a .oxoloquin alkaloid isola m the pJants of gellera *Berberis* and *Coptis*, produces protections against α arrhythmias animaJ and clω 1 a1s(p) Moreover Ber exerts 8t)y other beneficial effects eg, antiplatelet aggregation vasodilation protectt ve action against hypoxia mui tet ofωIngestive heart failure[3-5]. 1 acelluJar trline ω transmembrane potentiaW.W8S fu:t reported íD 1 5md the cellular electrophysiologic mechanism of Ber was

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lDten ωstudy e effects of Ber on L. peωlcium Cnnp.nhl \"UUCp(8 In guinea pig 'ventriωlar

MATERIALSAND METHODS

· pm m on of ven cularu Single m gl ventriωlar myocytes w by enz m :(10). Gui (nd waamgmu" g)were mned ld eirhe were ónsed in g oated (}1-+ee Tyr e'g solution. the aorta wasωnnu)ated a00 moun a LangendodIaPJ: 1S at31 't. A wi o1-+rode'g soluti for 5 min was follow by 10W 01+ (50 tJlDol. L-1) physiølogie solntj] ng 0.03 % ω enase and 1 % 00 . ueserwn umin (BSA) for 5 min. 'Il. veI cles wI eamin. cedand gently trl rated wi a E een me soluti 6min. ltw d mh a 2 μm nylon mesh ld gJ." Ial1y increas00 the of+ concentrati mω1.0 nnnol. L-1 - ly the cells wi :ed and ble striations were u 1e1 ts.

Cbetnicals and ωllutim Berberinehy 'ochl dew purcbased om Sigtlla CO and w dissolved in dis 100 wa ω make a 2.5 ßnDol.L-[1] :>k solutiω. BSA collagenase type II HEPES tntJnol1 UDl (t 7IC acidCSCl CSOMgA t and ne were ZL H ωan ProdωM dωna. 3-(N..ra.X 1 lino)prq nedonic acid' (MOPS) sputcba m B .o Biotech Co i6ωd or+ ?sωlution wω:

Lr22t 110 mom-IUI; was adJ1S: TEAOHω13 1 : d:222f Efzr: 3

adjusted

Measurement of .c::i/'t :CI .;. W _foa n a t M.ceJ)s placed in a 500µd dmMmmtMMmtnn! . '1'1" chamber w8S t 8011 2EP min-1 at 25 't. Mer J. ecmren weIe reC<X du tne whole– 11 hclarnp Edqm w a 1)8 h-clamp' ampl E (CEZ 23 NJhon Kol ß Japan) (10) _ rh ell s Wm 11 with vedω1 1 (PB–7 Nari ge 1 'o lapan) and had a resi B f 2 – 3 MO when filled .wi electrode M mal soluti Az S seal formation and' h rupture extemal solu dω Na+ fiee solution in

w also inactivated at tIEh of -40 mV and bl ked by TIX. K+ c ren were sl ed by substituting K+ by cs+ . dmn data uisit i ! stmp Wlll a α :ed with ρa 5.51 (Ax Instrument USA) nmning a personalα pu _

S 6ω All da were expressed as x s. The .iJJed *t*-test was usedωde mne s 686ωr sÌ ificance. To esti:mate the uency-del :ßdent efft of B βanalysis ɑf covariamce and e group *t*-test were u d.

RESUL 3

L-type ea2+αlrrent L-type Ca^{2+} currents in Fmea pig ven cul IJY y s was evoked by a depolarizing step pulse from e holding potential (E_b) of -40 mVωo mV at e uency of 1 Hz. le step pulse duration was 3 ms. Ber 10 and 30µmol .L-1 inhibi ak alIIpliude of feud hm 14MD · 247 pA (cantrol) ω978 204 pA (n =5 ceUs of 5 guinea pigs P <0.0 andω664 179pA (n =5 P < 0.01)res ctively (Fìg 1). inhibìtory effect was concentration-dφendent and was difficult to be recovered by washout wì control solution for 10 ID1D.

Current-voltage relationship of lea L and action of Ber Current-voltage (*1 – V)* curve of L type current was obtained by a number of dep ng s p pulses (3 ms) frQ111 e *Eh* of –40 mVωtest potentials ootween –30 mV and ωmV. The st s p pulses were delivered in 10-mV increments 1Ca.L was activated at –30 mV and the p k amplitude occurred at epo tial of 0 mV. Ber 10 and 30 µmol·L–1 upshi d e 1 – Vα1fVe e current mat •• of 0 mV was decreased 1 14.9::t3.2 pA/pF to 9.4 *3.0 p*A1 d ω 6.4J 1.1 F (ww=5 cells of 3 gu ikmZ (Fig 2) .

Frequency..dependency Ft uency-dependency was tested by chan19 e s mulating uencmω O.5 1 2 and 3 Hz. Each trajn uency n m 20 pulses. le interval between 2 trains of di rent uencies was 2 nl n. 1Ca.L was decreωedas e stimula 19 uency increased. After exposing to BeF 10µnol·L-1 1Ca.L was markedly reduced at each -equency. But the slope of the fr ency-depe dency Clifve was not changed by Ber Whidh was tested by analysis of covariance (Fig 3) .

ACtivatiOll and inactivation ldnetics of lea L The activation and inactivation CUIVes were E ed accordingωthe Bol m m uation: I/I_{max} = $1/\{1 + EXP[(V - Vo.s)1κ]\}$. Ber 10µmol.L-1 did not influence much the activation curve (half activation potential (V_0.5) from 15.3 mVω-17.2mV e s}ope factor (m 3.41 to 3.40 (n = 5 cel8 of 2 guinea pigs P > 0.05) . However lB er 10µnol. L-il i ed e $V_{0.s}$ from -27.8 mV tø -34.2 mV

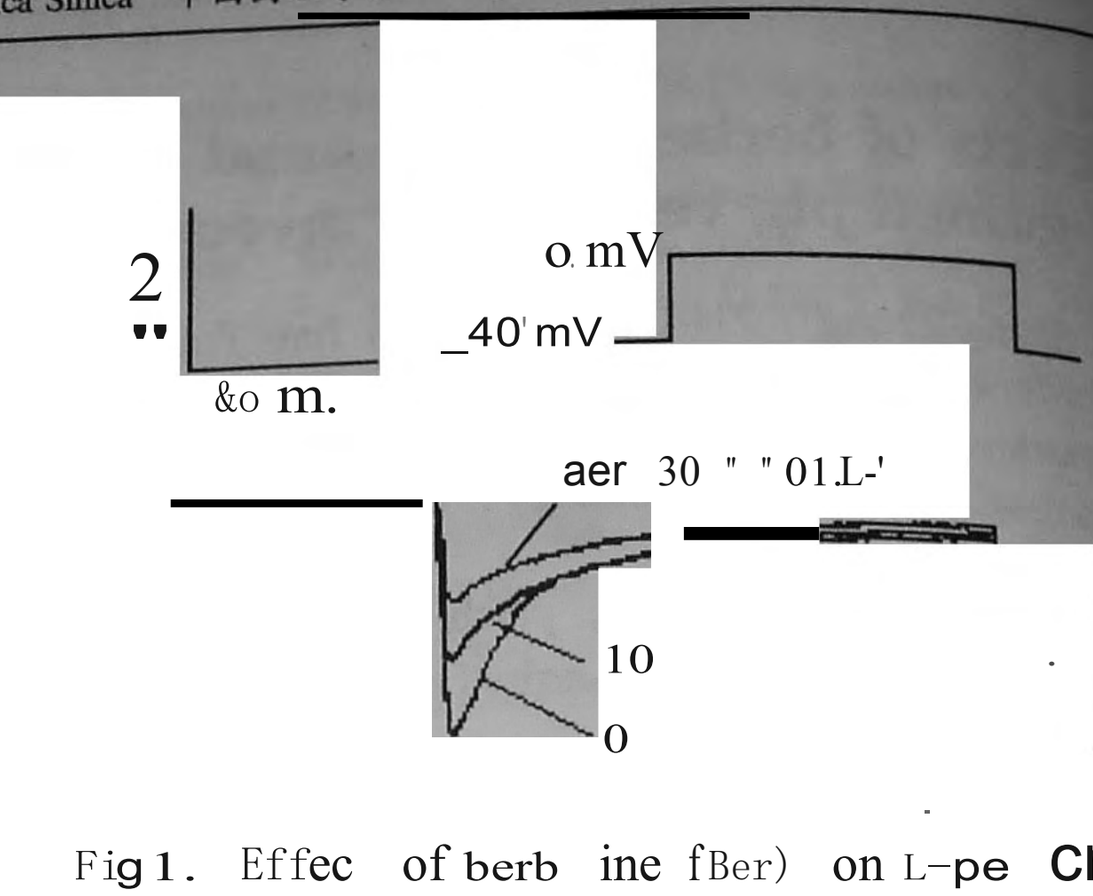

Fig 1. Effec of berb ine fBer) on L–pe cr+ . current in ventriɑdar Dly s' of guineag.

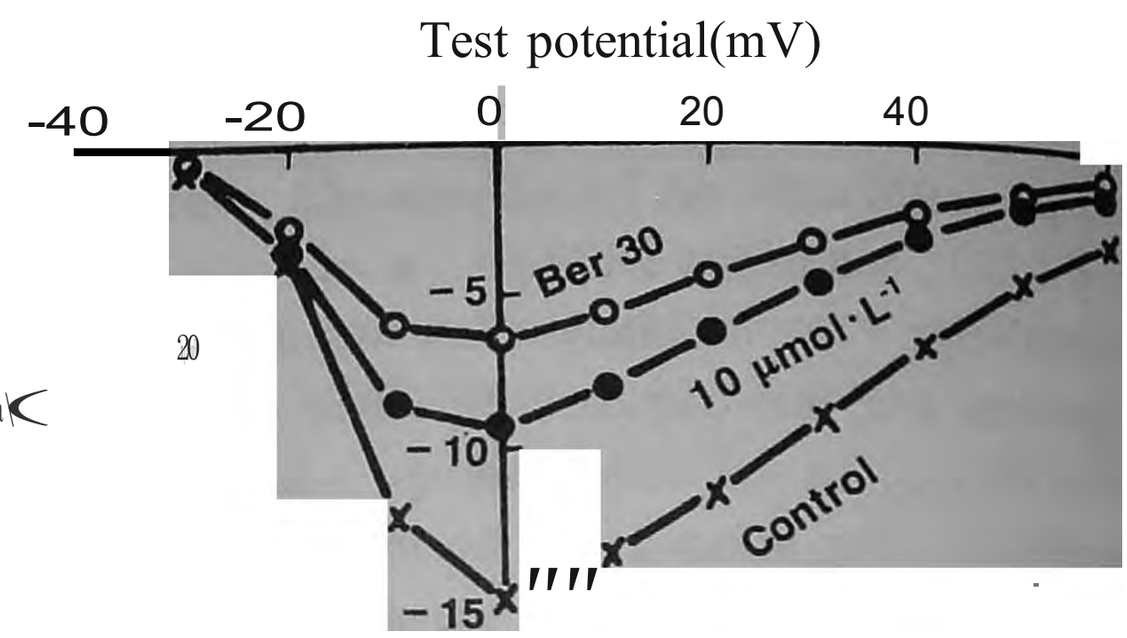

Fig 2. E tsof Ber on I-V rdation ip of L–type Ca^{2+} α Eth my tes (n :;; S cells of 3 g1 m pig) .

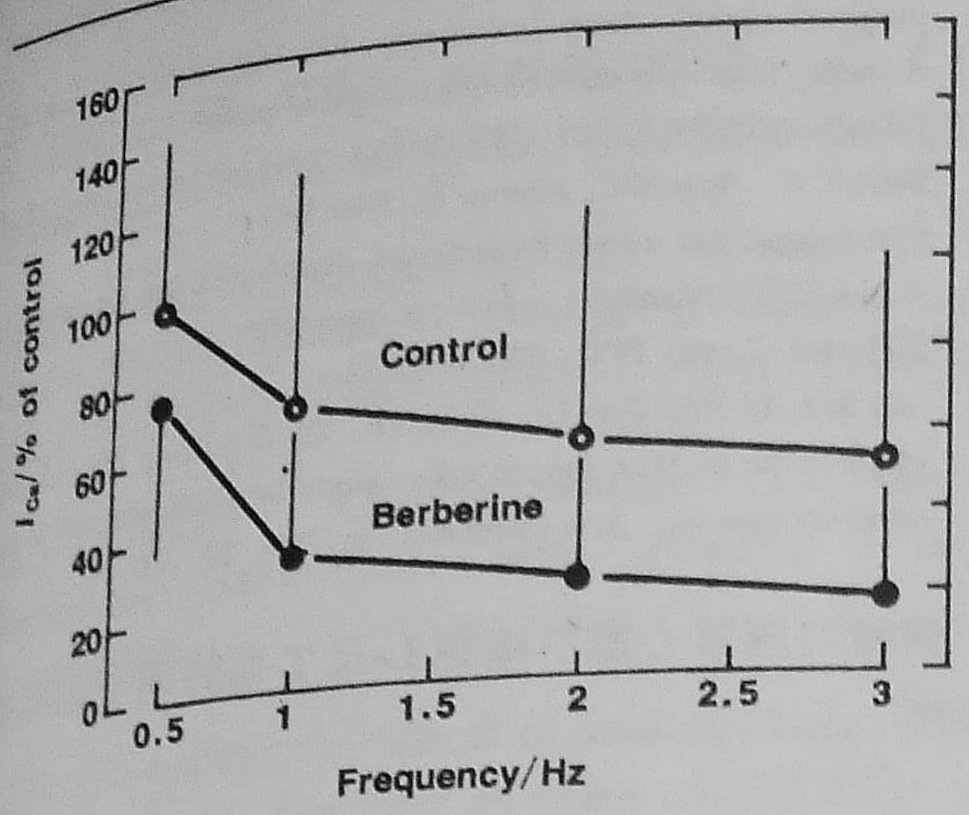

Fig 3. No use-dependent block of berberine on L-type Ca^{2+} channel in myocytes.

and κ from 9.22 to 13.03 ($n = 5$, $P < 0.01$) (Fig 4).

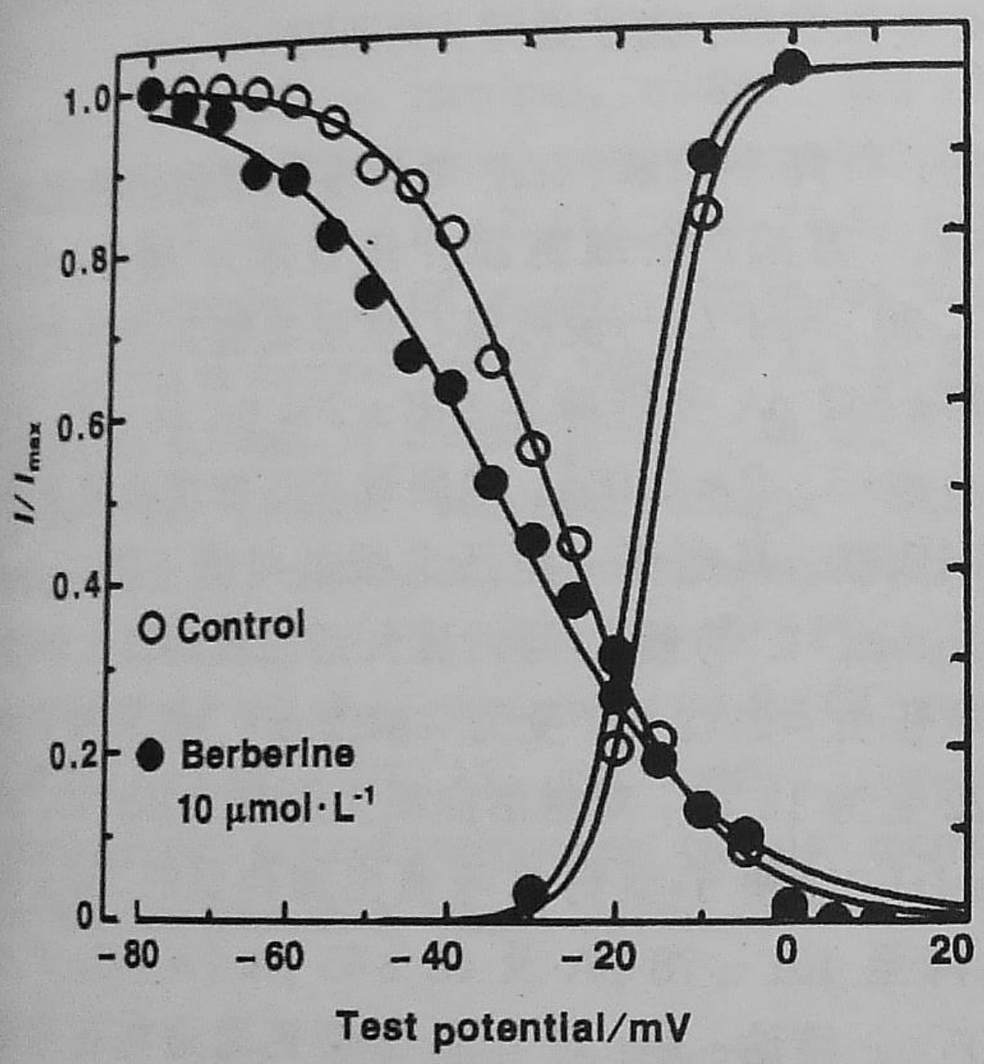

Fig 4. Effects of Ber on steady-state activation and inactivation kinetics of L-type Ca^{2+} current in myocytes.

$I_{Ca,T}$ $I_{Ca,T}$ was obtained[11]. The perfusing solution contained TTX 30 μmol·L^{-1} and nitrendipine 3 μmol·L^{-1} to block I_{Na} and $I_{Ca,L}$. Ber reduced the $I_{Ca,T}$. The peak amplitude of $I_{Ca,T}$ was decreased from 154 ± 80 pA to 101 ± 78 pA ($n = 8$, $P < 0.05$) with Ber 10 μmol·L^{-1}, and to 48 ± 18 pA with Ber 30 μmol·L^{-1} ($n = 8$ cells of 5 guinea pigs, $P < 0.05$) (Fig 5).

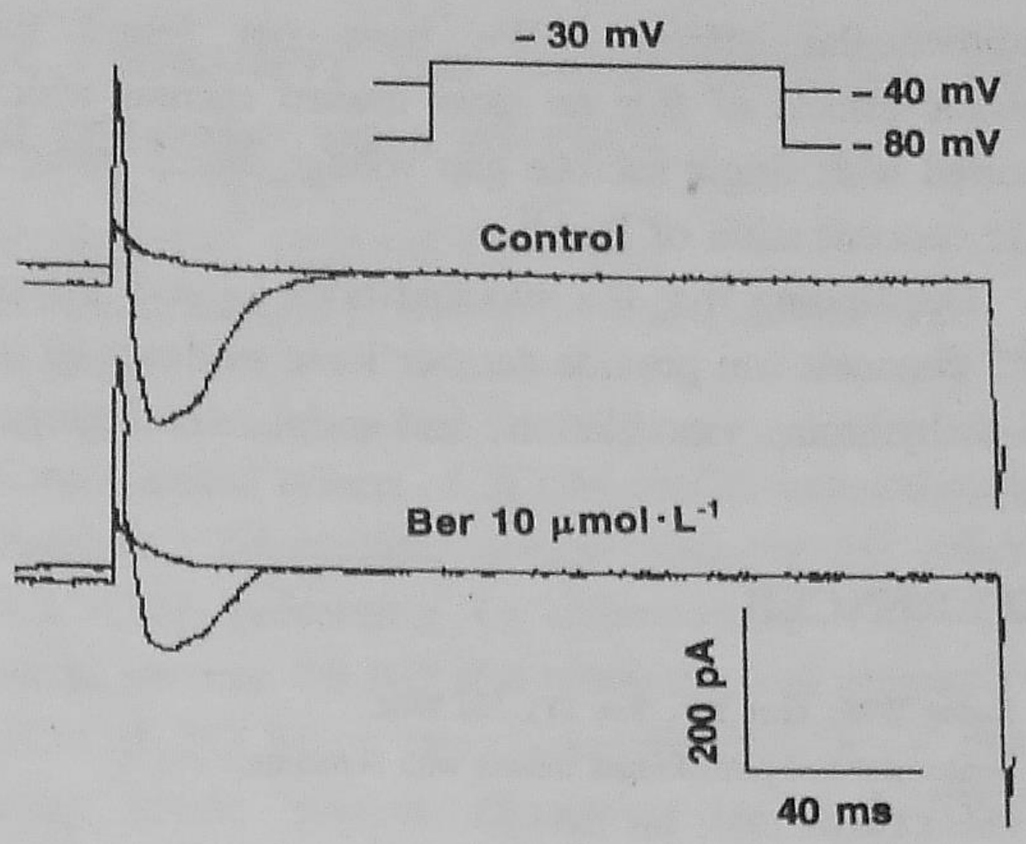

Fig 5. Effects of Ber on T-type Ca^{2+} current in myocytes.

DISCUSSION

Although the benificial effects of Ber on cardiovascular and other systems have been extensively investigated, the studies on its ionic mechanism are rare. The inhibitory effect of Ber on I_k was first reported in 1992[7] and recently confirmed by others[8, 9]. In this study, we found that Ber significantly decreased the L- and T-type Ca^{2+} currents. The inhibitory effect on $I_{Ca,L}$ was non-frequent dependency. The inactivation kinetics of $I_{Ca,L}$ were changed. It indicated that Ber blocked Ca^{2+} channel by its influence on the inactivation kinetics.

Two type calcium channels have been distinguished on the basis of differences in gating, pharmacological sensitivity in guinea pig ventricular cells. The physiological significance of L-type calcium channel is much more clear than that of T-type. It is related to the formation of action potential plateau, excitation-contraction coupling of muscle and the release of Ca^{2+} from sarcolemma reticulum. T-type calcium channels might be participated in the spontaneous activity of cells and some abnormal impulse formations. To eliminate the possibility of contamination by Na^+ current, we have worked in Na^+ free solution. This strategy has the additional virtue of disabling the electrogenic process of Na^+-Ca^{2+} exchange. The concentration of Ber we chose in this study was a low-to-middle one that could exert blocking actions on potassium currents and other

ωrdiovωcular e ectsi We have not found e bioh dωoof B slow inw d current d mrtd smglemcm ap vol e clamp usmg a toxic conωntration of Bef uJ

our lding at Ber bl ked 100 | Landτ'-type Ci+ ch 11 provide 8ltlO er ionic evidelll' f anti- "mua vasodHad011 andmtipiatdet aggega-tion.

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: (Ber)
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@ 30 ol.L-1J L
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pA (n =5P < 0.06)
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(10 lol. L--1) L
27.8 mv -34.2mv
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pA 101 '78 PA 48 45pA(nz8 p <
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