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Protein kinase C regulates glucose uptake and mRNA expression of glucose transporter ( ) 1 and in lactating bovine mammary epithelial cells

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ABSTRACT

The aim of this study was to determine the role of protein kinase in re ulatin lu ose uptake in la atin o ine mammary epithelial ells . The were ultured and treated with different on en trations of phor ol myristate a etate and n m the assi a i ator of for h ompared with the ells with no treatment and n of m si nifi antly stimulated the lu ose uptake of the whereas the lu ose uptake y the ells treated with the lowest and the hi hest amounts of and n m respe i ely did not show a si nifi ant differen e on sistently the m e pression of lu ose transporter *GLUT* and showed a similar pattern of in ease under the treatments of urthermore when the ells were pretreated with and *M* an inhi itor of for min efore e posed to n m the indu d lu ose uptake and *GLUT1* and *GLUT8* e pression were de eased y in a dose dependent man ner These results demonstrate that is in ol ed in the re ulation of lu ose uptake y and this fun ion may work at least partly throu h upre ulat in the e pression of *GLUT1* and *GLUT8*

Key words: o ine lu ose transporter mammary epithelial ell protein kinase

Short Communication

lu ose is an important su strate and ener y sour e in milk synthesis ar e amounts of lu ose are re uired to sustain la ation ant et al lu ose is taken up from the lood stream y mammary se re tory epithelial ells **MEC** throu h fa ilitati e lu ose transporters **GLUT** mainly T and possi ly T hao rotein kinase **PKC** is an important ell si nalin mole ule that modulates a ariety of ellular pro esses su h as se retion ene e pression proliferation differ entiation and mus e ontra tion ishi uka n addition it is well known that is a key re ulator in lu ose uptake in a ariety of ells Watson and es sin os n et al uiken et al ow e er whether re ulates lu ose uptake in is not known pression of proteins in eases in human mammary epithelial tissue from pu erty to pre nan y asso Wel n et al i ation of enhan es proliferation and sur i al rossoni et al and plays an important role in prola in indu ed milk protein synthesis in mouse arte et al urthermore han es in e pression or a i ity ha e een o ser ed durin mammary ar no enesis rtre er et al Therefore may also e an important re ulator in re ulatin lu ose uptake in The o e i e of the urrent study was to determine the role of in re ulation of lu ose transport and lu ose transporter ene e pression in la atin o ine mammary epithelial ells **BMEC**

The la atin were isolated hara eri ed and ultured as pre iously des i ed hao et al iu et al The ells were star ed with serum free medium for h and then treated with different on entrations and n m of phor ol myristate a etate **PMA** i ma t ouis a assi a i ator of for h and with different on entrations and *M* of er k ios en es armstadt ermany an inhi itor of for min efore e in treated with n m for another h The differen e in lu ose ontent in the ulture media efore and after the treatments determined y an en ymati olorin lu ose o idase pero idase assay method Tiffany et al and normali ed y total ell protein ontent was onsidered as the net lu ose uptake orsi et al

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T ol rea ent n itro en orp arls ad was used to isolate total from the ells The first strand of was trans i ed usin a re erse tran s iption kit Takara Tokyo apan The m a undan e of *GLUT1* and *GLUT8* was analy ed y uantitati e re erse trans iption usin reen as des i ed efore hao et al The rela ti e m han es of ea n ene were al ulated usin the T where T y e threshold method i ak and mitt en and normali ed to a in m in the same sample

ata were analy ed y one way V usin software ersion orp rmonk and un n s multiple ran e tests were used for multiple omparisons a n e periment was performed with to repli ates and repeated times usin pooled isolated from olstein la atin ows ata from representati e e periment is reported here i nifi ant differen s were onsidered at $P <$

n this study we used a a i ator and a inhi itor to study the role of in re ulation of lu ose uptake and *GLUT* e pression in hor ol myristate a etate is a diester of phor ol whi h has een widely used in iomedi l resear h to a i ate assi l os n et al oel et al e ause of its stru ural similarity to one of the natural a i ators of assi isoforms dia l ly erol ompared with the ontrol roup n m treatment of with and n of m si nifi antly in reased the ellular lu ose uptake $P <$ i ure whereas the lu ose uptake in the ells treated with either the lowest on entration n m or the hi hest on entration n m of did not show a si nifi ant differen e P i ure n addition when were treated with a pan inhi itor o erts et al uyen arese and a an at the on entrations of and M efore treatment n m a i ated lu ose uptake was si nifi antly inhi ited y at a on entration of M or hi her $P <$ i ure These results demonstrate a re ulatory role of in lu ose transport in onsistent with pre ious o ser ations in adipo tes and intestinal ells i s et al a e et al hen and arr urthermore we found that in re u lates m e pression of *GLUT1* and *GLUT8* the main lu ose transporters e pressed in the mammary land hao et al n parti ular and n of m si nifi antly in reased the m e pression of *GLUT1* and *GLUT8* in $P <$ i ure and ompared with the ontrol n of m whereas and n m had no effe P These o ser ations are in line with the report

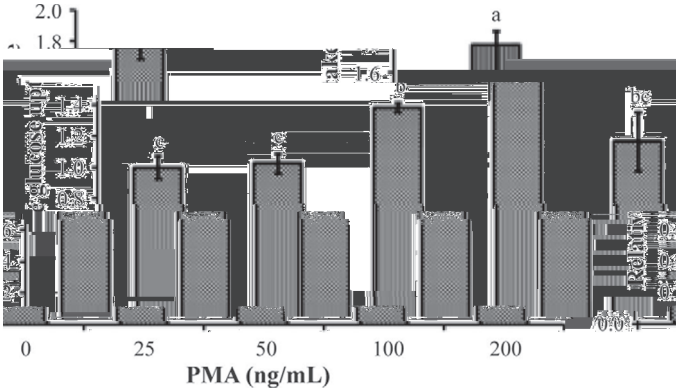

Figure 1. lu ose uptake in o ine mammary epithelial ells treated with different on entrations of phor ol myristate a etate The lu ose uptake in the ontrol ells without treatment n m is assi ned a alue of Values with different letters a are si nifi antly different $P <$ n rror ars indi ate the

that *GLUT1* m le els were in reased y a i ation of in o ine rain apillary endothelial ells ar rell et al and su est that the a i ated lu ose uptake in may e mediated y enhan d e pression of *GLUT1* and *GLUT8* n addition *GLUT8* m was hi her at n m of ompared with n m $P <$ i ure whereas no dif feren e was seen in *GLUT1* e pression etween these roups P i ure imilar to the lu ose uptake the indu ed *GLUT1* m e pression in was si nifi antly inhi ited y at a on entration of M or hi her $P <$ i ure whereas the indu ed *GLUT8* m e pres sion was si nifi antly inhi ited y at a

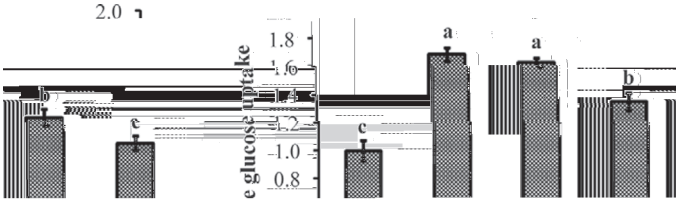

Figure 2. ffe of an inhi itor of protein kinase on phor ol myristate a etate indu ed lu ose uptake in o ine mammary epithelial ells ells were in u ated in media with different on entrations of and M for min and then treated with or without n m The lu ose uptake in the roup without and treat ments is assi ned a alue of Values with different letters a are si nifi antly different $P <$ n rror ars indi ate the

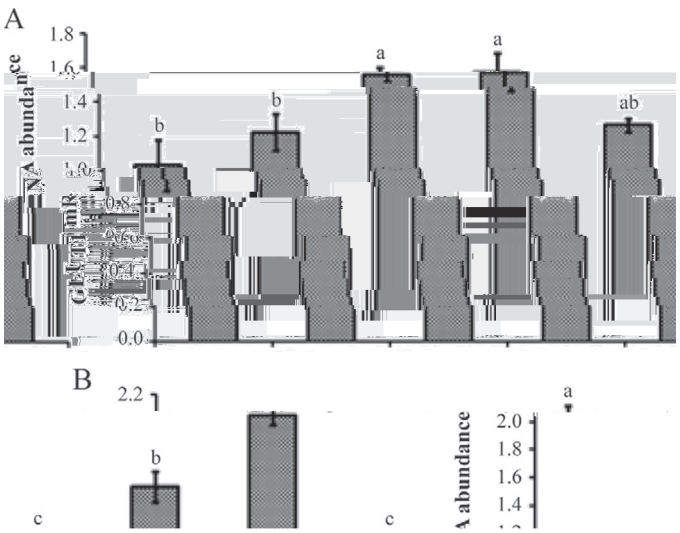

Figure 3. The m a undan e of lu se transporter *GLUT1* and *GLUT8* enes in o ine mammary epithelial ells treat ed with different on entrations of phor ol myristate a etate , The m le els of *GLUT1* and *GLUT8* in the ells without , treatment are assi ned alues of Values with different letters a are si nifi antly different $P <$ n rror ars indi ate the

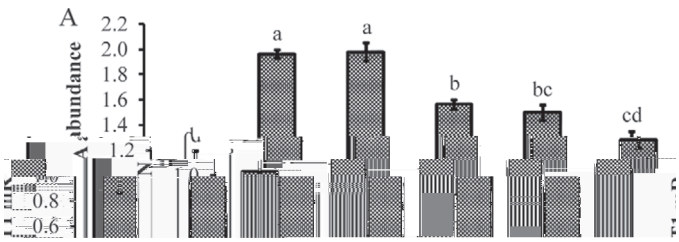

Figure 4. ffe of an inhi itor of protein kinase on phor ol myristate a etate , indu ed lu se trans porter *GLUT1* and *GLUT8* m e pression in o ine mammary epithelial ells ells were in u ated in media with different on entra tions of and *M* for min and then treated with or without , n m The *GLUT1* and *GLUT8* m le els in the roup without , and treatments were assi ned alues of Values with different letters a d are si nifi antly different $P <$ n rror ars indi ate the

on entration of *M* or hi her $P <$ i ure t appeared that the m e pression of *GLUT8* in , may e more sensiti e to a i ation than the e pression of *GLUT1*

re ious studies ha e shown that a i ation indu es a i ator protein **AP-2** mediated tran s iptional a i ation ma awa et al yman et al oth phor ol ester response elements and indin sites ha e een identified in the rat *GLUT1* ene in li er ells ehroo and smail ei i The *GLUT1* ene is hi hly onser ed a oss mammalian spe es hao and eatin and the rat phor ol ester response element and human element indin sites ha e een found in the o ine *GLUT1* ene Therefore it is likely that may a t on these sites dire ly or indire ly to a i ate T e pression in , The o ine *GLUT8* ene has not een well ara eri ed thus it is not known whether these elements are present in o ine *GLUT8* ene

We annot rule out the possi ility that may stimulate lu se uptake y the me anisms other than enhan n *GLUT* m e pression os n et al reported that , stimulates lu se uptake in T adipo tes y in reasin the T protein ontent in the total ell homo enate , oreo er the translo ations of T i s et al os n et al and T in rat adipo tes tandaert et al otani et al Watson and essin and T in rat intestinal ells hen and arr were o ser ed in re ulated lu se transport Therefore it is possi le that may also re ulate protein translation translo ation or transport kineti s of T in , to enhan e lu se uptake n summary this study pro ided e iden e that may play a role in the re ulation of lu se uptake and e pression of *GLUT1* and *GLUT8* in ,

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REFERENCES

orsi am eroni sani o oni Vi iani , onari , e m ro i , unno Tamanini and eren eptin does not seem to influen e lu se uptake y o ine mammary e plants hysiol harma ol ehroo and smail ei i ual ontrol of *glut1* lu se transporter ene e pression y hypo ia and y inhi ition of o ida ti e phosphorylation iol hem osh a uine pan , Willems lthaar an ennes , aassen Ta k , ermus and weep e ulation of T mediated lu se uptake y intera ions in T adipo tes io hem osh a uine , ake pan lthaar h rmann , aassen , ermus , Wil lems and weep nhi ition of protein kinase in reases lu se uptake in T adipo tes throu h ele ated e pression of lu se transporter at the plasma mem rane , ol ndo inol ant Trout iao and urdie , ilk syn theti response of the o ine mammary land to an in rease in the lo al on entration of arterial lu se airy arese V and , a an , eta oli fun ions of atypi al protein kinase ood and ad as defined y nutritional sta tus m hysiol ndo inol , eta arrell oado and W , ardrid e nhan d T lu se transporter and toskeleton ene e pression in ultured o ine rain apillary endothelial ells after treatment with phor ol esters and serum rain es , ol rain es is s , , alderhead olman and W ould hor ol ester only partially mimi s the effe s of insulin on lu se transport and lu se transporter distri ution in T adipo tes io hem oel , akkar ran s and e ter hor ol esters tru ure iolo i al a i ity and to i ty in animals nt To i ol rossoni V al o , a aniet de ier offe and rtre er rotein kinase enhan s pro liferation and sur i al of murine mammary ells , ol ar no yman , om earl er and , oodman n element a s syner isti ally with the y i , and phor ol ester indu le enhan er of the human proenkephalin ene , ol ell iol ma awa , hiu and , arin Trans iption fa or mediates indu ion y two different si nal transdu ion pathways rotein kinase and , ell otani W awa , , atsumoto T itamura akaue ino , iyake W ano kimoto hno and , a su a e uirement of atypi al protein kinase for insulin stimulation of lu se uptake ut not for kt a i ation in T adipo tes , ol ell iol iu hao and iu ffe s of lu se a aila ility on e pression of the key enes in ol ed in synthesis of milk fat la tose and lu se meta olism in o ine mammary epithelial ells o e i ak and T mitt en nalysis of relati e ene e pression data usin real time uantitati e and the method , ethods uiken uwens a ets , an der on W oumans W uwenk onen and lat ermissi e a ion of protein kinase in insulin indu d and T translo ation in ardia myo tes ndo inol , arte , T, eyer ta el tandke aken a ro and ynes rotein kinase and mammary ell differentiation n ol ement of protein kinase in the indu ion of asein e pression ell rowth iffer asso Wel h Versto sek and , p lterations in the e pression and lo ali ation of protein kinase isoforms durin mammary land differentiation ur ell iol a e T iddle and hepherd hor ol esters stimulate phosphatidylinositol trisphosphate produ ion in T adipo tes mpli ations for stimulation of lu se trans port io hem uyen T Tar etin n o er iew of small mole ule inhi itors nti an er ents, ed hem ishi uka tudies and perspe i es of the protein kinase en e o erts aworth and, kiran ffe s of isin dolylmaleimide inhi itors on p a i ity *in vitro* and in adult entri ular myo tes r harma ol tandaert , alloway arnam andyopadhyay , os t and V arese rotein kinase as a down stream effe or of phosphatidylinositol kinase durin insulin stimulation in rat adipo tes otential role in lu se transport iol hem Tiffany T , ansen urtis erton and ott n ymati kineti rate and end point analyses of su strate y use of a e fast analy er lin hem rtre er , a aniet and al de ier offe ontri ution of indi idual isoforms to reast an er pro res sion , ife Watson T and essin u ellular ompartmentali a tion and traffi kin of the insulin responsi e lu se transporter T p ell es hao iolo y of lu se transport in the mammary land , ammary land iol eoplasia http d doi or s hao W T i on and ennelly o ali ation and ene e pression of lu se transporters in o ine mammary land omp io hem hysiol io hem, ol iol hao and eatin pression and re ulation of lu se transporters in the o ine mammary land airy uppl hao , iller Wall hen on , e ille and T , adden o ine lu se transporter T lonin e pression and de elopmental re ulation in mammary land io him iophys a hao iu Wan , , hou and iu ffe s of lu se a aila ility on lu se transport in o ine mam mary epithelial ells nimal hao iu , , hou and iu sta lishment and hara eri ation of a la atin o ine mammary epithelial ell model for the study of milk synthesis ell iol nt hen and , arr Translo ation of transfe ed T to the api al mem rane in rat intestinal tal u mi nd s Tw Td