Short communication: Protein kinase C regulates glucose uptake and mRNA expression of glucose transporter (*GLUT*) 1 and *GLUT8* in lactating bovine mammary epithelial cells

K. Zhao,*† H.-Y. Liu,*¹ F.-Q. Zhao,*‡ and J.-X. Liu*¹

*Institute of Dairy Science, MOE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou 310058, P. R. China

†Shaanxi Institute of Zoology, 88 Xingqing Road, Xi'an 710032, P. R. China

‡Laboratory of Lactation and Metabolic Physiology, Department of Animal Science, University of Vermont, Burlington 05405

ABSTRACT

The aim of this study was to determine the role of protein kinase C (PKC) in regulating glucose uptake in lactating bovine mammary epithelial cells (BMEC). The BMEC were cultured and treated with different concentrations of phorbol 12-myristate 13-acetate (PMA; 0, 10, 25, 50, 100, and 200 ng/mL), the classic activator of PKC, for 48 h. Compared with the cells with no PMA treatment, 50 and 100 ng of PMA/mL significantly stimulated the glucose uptake of the BMEC, whereas the glucose uptake by the cells treated with the lowest and the highest amounts of PMA (25 and 200 ng/mL, respectively) did not show a significant difference. Consistently, the mRNA expression of glucose transporter (*GLUT*) 1 and 8 showed a similar pattern of increase under the treatments of PMA. Furthermore, when the cells were pretreated with GF1090203X (0, 0.25, 0.5, 1, and 2 μ M), an inhibitor of PKC, for 30 min before exposed to PMA (50 ng/mL), the PMA-induced glucose uptake and *GLUT1* and *GLUT8* expression were decreased by GF1090203X in a dose-dependent manner. These results demonstrate that PKC is involved in the regulation of glucose uptake by BMEC, and this function may work, at least partly, through upregulating the expression of *GLUT1* and *GLUT8*.

Key words: bovine, glucose transporter, mammary epithelial cell, protein kinase C

Short Communication

Glucose is an important substrate and energy source in milk synthesis. Large amounts of glucose are required to sustain lactation (Cant et al., 2002). Glucose is taken up from the blood stream by mammary secretory epithelial cells (MEC) through facilitative glucose

transporters (*GLUT*), mainly *GLUT1* and possibly *GLUT8* (Zhao, 2014).

Protein kinase C (PKC) is an important cell signaling molecule that modulates a variety of cellular processes, such as secretion, gene expression, proliferation, differentiation, and muscle contraction (Nishizuka, 1986). In addition, it is well known that PKC is a key regulator in glucose uptake in a variety of cells (Watson and Pessin, 2001; Bosch et al., 2004; Luiken et al., 2009). However, whether PKC regulates glucose uptake in MEC is not known. Expression of PKC proteins increases in human mammary epithelial tissue from puberty to pregnancy (Masso-Welch et al., 1999). Activation of PKC enhances proliferation and survival (Grossoni et al., 2007) and plays an important role in prolactin-induced milk protein synthesis in mouse MEC (Marte et al., 1994). Furthermore, changes in PKC expression or activity have been observed during mammary carcinogenesis (Urtreger et al., 2012). Therefore, PKC may also be an important regulator in regulating glucose uptake in MEC. The objective of the current study was to determine the role of PKC in regulation of glucose transport and glucose transporter gene expression in lactating bovine mammary epithelial cells (BMEC).

The lactating BMEC were isolated, characterized, and cultured as previously described (Zhao et al., 2010; Liu et al., 2013). The cells were starved with serum-free medium for 12 h and then treated with (1) different concentrations (0, 10, 25, 50, 100, and 200 ng/mL) of phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, MO), a classic activator of PKC, for 48 h and (2) with different concentrations (0, 0.25, 0.5, 1, and 2 μ M) of GF1090203X (Merck Biosciences, Darmstadt, Germany), an inhibitor of PKC, for 30 min before being treated with PMA (50 ng/mL) for another 48 h. The difference in glucose content in the culture media before and after the treatments, determined by an enzymatic coloring glucose oxidase/peroxidase assay method (Tiffany et al., 1972) and normalized by total cell protein content, was considered as the net glucose uptake (Accorsi et al., 2005).

Received December 18, 2013.

Accepted March 20, 2014.

¹Corresponding authors: hyliu@zju.edu.cn and liujx@zju.edu.cn

TRIzol reagent (Invitrogen Corp., Carlsbad, CA) was used to isolate total RNA from the cells. The first strand of cDNA was transcribed using a reverse-transcription PCR kit (Takara, Tokyo, Japan). The mRNA abundance of *GLUT1* and *GLUT8* was analyzed by quantitative reverse-transcription PCR using SYBR Green as described before (Zhao et al., 2012). The relative mRNA changes of each gene were calculated using the $2^{-\Delta\Delta CT}$ (where CT = cycle threshold) method (Livak and Schmittgen, 2001) and normalized to β -actin mRNA in the same sample.

Data were analyzed by one-way ANOVA using SPSS software (version 16.0; IBM Corp., Armonk, NY), and Duncan's multiple range tests were used for multiple comparisons. Each experiment was performed with 3 to 6 replicates and repeated 3 times using pooled BMEC isolated from 3 Holstein lactating cows. Data from 1 representative experiment is reported here. Significant differences were considered at $P < 0.05$.

In this study, we used a PKC activator (PMA) and a PKC inhibitor (GF1090203X) to study the role of PKC in regulation of glucose uptake and *GLUT* expression in BMEC. Phorbol 12-myristate 13-acetate is a diester of phorbol, which has been widely used in biomedical research to activate classical PKC (Bosch et al., 2004; Goel et al., 2007) because of its structural similarity to one of the natural activators of classic PKC isoforms, diacylglycerol. Compared with the control group (0 ng/mL), treatment of BMEC with 50 and 100 ng of PMA/mL significantly increased the cellular glucose uptake ($P < 0.05$; Figure 1), whereas the glucose uptake in the cells treated with either the lowest concentration (25 ng/mL) or the highest concentration (200 ng/mL) of PMA did not show a significant difference ($P > 0.05$; Figure 1). In addition, when BMEC were treated with a pan-PKC inhibitor, GF1090203X (Roberts et al., 2005; Nguyen, 2008; Farese and Sajan, 2010), at the concentrations of 0.25, 0.5, 1, and 2 μM before PMA treatment (50 ng/mL), PMA-activated glucose uptake was significantly inhibited by GF1090203X at a concentration of 0.5 μM or higher ($P < 0.05$; Figure 2). These results demonstrate a regulatory role of PKC in glucose transport in BMEC, consistent with previous observations in adipocytes and intestinal cells (Gibbs et al., 1991; Navé et al., 1996; Zheng and Sarr, 2012).

Furthermore, we found that in BMEC, PKC regulates mRNA expression of *GLUT1* and *GLUT8*, the main glucose transporters expressed in the mammary gland (Zhao et al., 1996, 2004). In particular, 50 and 100 ng of PMA/mL significantly increased the mRNA expression of *GLUT1* and *GLUT8* in BMEC ($P < 0.05$; Figure 3A and 3B) compared with the control (0 ng of PMA/mL), whereas 25 and 200 ng/mL had no effect ($P > 0.05$). These observations are in line with the report

Figure 1. Glucose uptake in bovine mammary epithelial cells treated with different concentrations of phorbol 12-myristate 13-acetate (PMA). The glucose uptake in the control cells without PMA treatment (0 ng/mL) is assigned a value of 1.0. Values with different letters (a–c) are significantly different ($P < 0.05$; $n = 6$). Error bars indicate the SE.

that *GLUT1* mRNA levels were increased by activation of PKC in bovine brain capillary endothelial cells (Farrell et al., 1992) and suggest that the PKC-activated glucose uptake in BMEC may be mediated by enhanced expression of *GLUT1* and *GLUT8*. In addition, *GLUT8* mRNA was higher at 100 ng/mL of PMA compared with 50 ng/mL ($P < 0.05$; Figure 3B), whereas no difference was seen in *GLUT1* expression between these 2 groups ($P > 0.05$; Figure 3A). Similar to the glucose uptake, the PMA-induced *GLUT1* mRNA expression in BMEC was significantly inhibited by GF1090203X at a concentration of 0.5 μM or higher ($P < 0.05$; Figure 4A), whereas the PMA-induced *GLUT8* mRNA expression was significantly inhibited by GF1090203X at a

Figure 2. Effect of GF1090203X, an inhibitor of protein kinase C, on phorbol 12-myristate 13-acetate (PMA)-induced glucose uptake in bovine mammary epithelial cells. Cells were incubated in media with different concentrations of GF1090203X (0, 0.25, 0.5, 1, and 2 μM) for 30 min and then treated with or without PMA (50 ng/mL). The glucose uptake in the group without PMA and GF1090203X treatments is assigned a value of 1.0. Values with different letters (a–c) are significantly different ($P < 0.05$; $n = 6$). Error bars indicate the SE.

Figure 3. The mRNA abundance of glucose transporter 1 (*GLUT1*; A) and *GLUT8* (B) genes in bovine mammary epithelial cells treated with different concentrations of phorbol 12-myristate 13-acetate (PMA). The mRNA levels of *GLUT1* and *GLUT8* in the cells without PMA treatment are assigned values of 1.0. Values with different letters (a–c) are significantly different ($P < 0.05$; $n = 3$). Error bars indicate the SE.

concentration of 0.25 μM or higher ($P < 0.05$; Figure 4B). It appeared that the mRNA expression of *GLUT8* in BMEC may be more sensitive to PKC activation than the expression of *GLUT1*.

Previous studies have shown that PKC activation induces activator protein-2 (AP-2)-mediated transcriptional activation (Imagawa et al., 1987; Hyman et al., 1989). Both phorbol ester response elements and AP-2 binding sites have been identified in the rat *GLUT1* gene in liver cells (Behrooz and Ismail-Beigi, 1997). The *GLUT1* gene is highly conserved across mammalian species (Zhao and Keating, 2007), and the rat phorbol ester response element and human AP-2 element binding sites have been found in the bovine *GLUT1* gene. Therefore, it is likely that PKC may act on these sites directly or indirectly to activate *GLUT1* expression in BMEC. The bovine *GLUT8* gene has not been well characterized; thus, it is not known whether these elements are present in bovine *GLUT8* gene.

We cannot rule out the possibility that PKC may stimulate glucose uptake by the mechanisms other

Figure 4. Effect of GF1090203X, an inhibitor of protein kinase C, on phorbol 12-myristate 13-acetate (PMA)-induced glucose transporter 1 (*GLUT1*) and *GLUT8* mRNA expression in bovine mammary epithelial cells. Cells were incubated in media with different concentrations of GF1090203X (0, 0.25, 0.5, 1, and 2 μM) for 30 min and then treated with or without PMA (50 ng/mL). The *GLUT1* and *GLUT8* mRNA levels in the group without PMA and GF1090203X treatments were assigned values of 1.0. Values with different letters (a–d) are significantly different ($P < 0.05$; $n = 3$). Error bars indicate the SE.

than enhancing *GLUT* mRNA expression. Bosch et al. (2003) reported that PMA stimulates glucose uptake in 3T3-L1 adipocytes by increasing the *GLUT1* protein content in the total cell homogenate. Moreover, the translocations of *GLUT1* (Gibbs et al., 1991; Bosch et al., 2003) and *GLUT4* in rat adipocytes (Standaert et al., 1997; Kotani et al., 1998; Watson and Pessin, 2001) and *GLUT2* in rat intestinal IEC-6 cells (Zheng and Sarr, 2012) were observed in PKC-regulated glucose transport. Therefore, it is possible that PKC may also regulate protein translation, translocation, or transport kinetics of *GLUT* in BMEC to enhance glucose uptake. In summary, this study provided evidence that PKC may play a role in the regulation of glucose uptake and expression of *GLUT1* and *GLUT8* in BMEC.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (Beijing, China; 30901034) and the earmarked fund for the China Agricultural Research System (CARS-37).

REFERENCES

- Accorsi, P. A., M. Gamberoni, G. Isani, N. Govoni, R. Viggiani, M. Monari, M. De Ambrogi, A. Munno, C. Tamanini, and E. Seren. 2005. Leptin does not seem to influence glucose uptake by bovine mammary explants. *J. Physiol. Pharmacol.* 56:689–698.
- Behrooz, A., and F. Ismail-Beigi. 1997. Dual control of *glut1* glucose transporter gene expression by hypoxia and by inhibition of oxidative phosphorylation. *J. Biol. Chem.* 272:5555–5562.
- Bosch, R. R., M. Bazuine, P. N. Span, P. H. G. M. Willems, A. J. Olthaar, H. van Rennes, J. A. Maassen, C. J. Tack, A. R. M. M. Hermus, and C. G. J. Sweep. 2004. Regulation of GLUT1-mediated glucose uptake by PKC α -PKC β_{II} interactions in 3T3-L1 adipocytes. *Biochem. J.* 384:349–355.
- Bosch, R. R., M. Bazuine, M. M. Wake, P. N. Span, A. J. Olthaar, A. Schürmann, J. A. Maassen, A. R. M. M. Hermus, P. H. G. M. Willems, and C. G. J. Sweep. 2003. Inhibition of protein kinase C β_{II} increases glucose uptake in 3T3-L1 adipocytes through elevated expression of glucose transporter 1 at the plasma membrane. *Mol. Endocrinol.* 17:1230–1239.
- Cant, J. P., D. R. Trout, F. Qiao, and N. G. Purdie. 2002. Milk synthetic response of the bovine mammary gland to an increase in the local concentration of arterial glucose. *J. Dairy Sci.* 85:494–503.
- Farese, R. V., and M. P. Sajan. 2010. Metabolic functions of atypical protein kinase C: “Good” and “bad” as defined by nutritional status. *Am. J. Physiol. Endocrinol. Metab.* 298:E385–E394.
- Farrell, C. R., R. J. Boado, and W. M. Pardridge. 1992. Enhanced GLUT1 glucose transporter and cytoskeleton gene expression in cultured bovine brain capillary endothelial cells after treatment with phorbol esters and serum. *Brain Res. Mol. Brain Res.* 15:221–226.
- Gibbs, E. M., D. M. Calderhead, G. D. Holman, and G. W. Gould. 1991. Phorbol ester only partially mimics the effects of insulin on glucose transport and glucose-transporter distribution in 3T3-L1 adipocytes. *Biochem. J.* 275:145–150.
- Goel, G., H. P. S. Makkar, G. Francis, and K. Becker. 2007. Phorbol esters: Structure, biological activity, and toxicity in animals. *Int. J. Toxicol.* 26:279–288.
- Grossoni, V. C., K. B. Falbo, M. G. Kazanietz, E. D. B. de Kier Joffé, and A. J. Urtreger. 2007. Protein kinase C δ enhances proliferation and survival of murine mammary cells. *Mol. Carcinog.* 46:381–390.
- Hyman, S. E., M. Comb, J. Pearlberg, and H. M. Goodman. 1989. An AP-2 element acts synergistically with the cyclic AMP- and phorbol ester-inducible enhancer of the human proenkephalin gene. *Mol. Cell. Biol.* 9:321–324.
- Imagawa, M., R. Chiu, and M. Karin. 1987. Transcription factor AP-2 mediates induction by two different signal transduction pathways: Protein kinase C and cAMP. *Cell* 51:251–260.
- Kotani, K., W. Ogawa, M. Matsumoto, T. Kitamura, H. Sakaue, Y. Hino, K. Miyake, W. Sano, K. Akimoto, S. Ohno, and M. Kasuga. 1998. Requirement of atypical protein kinase C α for insulin stimulation of glucose uptake but not for Akt activation in 3T3-L1 adipocytes. *Mol. Cell. Biol.* 18:6971–6982.
- Liu, H., K. Zhao, and J. Liu. 2013. Effects of glucose availability on expression of the key genes involved in synthesis of milk fat, lactose and glucose metabolism in bovine mammary epithelial cells. *PLoS ONE* 8:e66092.
- Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–408.
- Luiken, J. J. F. P., D. M. Ouwens, D. D. J. Habets, G. C. M. van der Zon, W. A. Coumans, R. W. Schwenk, A. Bonen, and J. F. C. Glatz. 2009. Permissive action of protein kinase C- ζ in insulin-induced CD36- and GLUT4 translocation in cardiac myocytes. *J. Endocrinol.* 201:199–209.
- Marte, B. M., T. Meyer, S. Stabel, G. J. R. Standke, S. Jaken, D. Fabbro, and N. E. Hynes. 1994. Protein kinase C and mammary cell differentiation: Involvement of protein kinase C α in the induction of β -casein expression. *Cell Growth Differ.* 5:239–247.
- Masso-Welch, P. A., G. Verstovsek, and M. M. Ip. 1999. Alterations in the expression and localization of protein kinase C isoforms during mammary gland differentiation. *Eur. J. Cell Biol.* 78:497–510.
- Navé, B. T., K. Siddle, and P. R. Shepherd. 1996. Phorbol esters stimulate phosphatidylinositol 3, 4, 5-trisphosphate production in 3T3-L1 adipocytes: Implications for stimulation of glucose transport. *Biochem. J.* 318:203–205.
- Nguyen, T. L. 2008. Targeting RSK: An overview of small molecule inhibitors. *Anticancer Agents Med. Chem.* 8:710–716.
- Nishizuka, Y. 1986. Studies and perspectives of the protein kinase C. *Science* 233:305–312.
- Roberts, N. A., R. S. Haworth, and M. Avkiran. 2005. Effects of bisindolylmaleimide PKC inhibitors on p90^{RSK} activity *in vitro* and in adult ventricular myocytes. *Br. J. Pharmacol.* 145:477–489.
- Standaert, M. L., L. Galloway, P. Karnam, G. Bandyopadhyay, J. Moscat, and R. V. Farese. 1997. Protein kinase C- ζ as a downstream effector of phosphatidylinositol 3-kinase during insulin stimulation in rat adipocytes. Potential role in glucose transport. *J. Biol. Chem.* 272:30075–30082.
- Tiffany, T. O., J. M. Jansen, C. A. Burtis, J. B. Overton, and C. D. Scott. 1972. Enzymatic kinetic rate and end-point analyses of substrate, by use of a GeMSAEC fast analyzer. *Clin. Chem.* 18:829–840.
- Urtreger, A. J., M. G. Kazanietz, and E. D. Bal de Kier Joffé. 2012. Contribution of individual PKC isoforms to breast cancer progression. *IUBMB Life* 64:18–26.
- Watson, R. T., and J. E. Pessin. 2001. Subcellular compartmentalization and trafficking of the insulin-responsive glucose transporter, GLUT4. *Exp. Cell Res.* 271:75–83.
- Zhao, F.-Q. 2014. Biology of glucose transport in the mammary gland. *J. Mammary Gland Biol. Neoplasia* 19:3–17. <http://dx.doi.org/10.1007/s10911-013-9310-8>.
- Zhao, F.-Q., W. T. Dixon, and J. J. Kennelly. 1996. Localization and gene expression of glucose transporters in bovine mammary gland. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 115:127–134.
- Zhao, F.-Q., and A. F. Keating. 2007. Expression and regulation of glucose transporters in the bovine mammary gland. *J. Dairy Sci.* 90(E. Suppl.):E76–E86.
- Zhao, F.-Q., P. J. Miller, E. H. Wall, Y.-C. Zheng, B. Dong, M. C. Neville, and T. B. McFadden. 2004. Bovine glucose transporter GLUT8: Cloning, expression, and developmental regulation in mammary gland. *Biochim. Biophys. Acta* 1680:103–113.
- Zhao, K., H. Y. Liu, H. F. Wang, M. M. Zhou, and J. X. Liu. 2012. Effect of glucose availability on glucose transport in bovine mammary epithelial cells. *Animal* 6:488–493.
- Zhao, K., H.-Y. Liu, M.-M. Zhou, and J.-X. Liu. 2010. Establishment and characterization of a lactating bovine mammary epithelial cell model for the study of milk synthesis. *Cell Biol. Int.* 34:717–721.
- Zheng, Y., and M. G. Sarr. 2012. Translocation of transfected GLUT2 to the apical membrane in rat intestinal IEC-6 cells. *Dig. Dis. Sci.* 57:1203–1212.