Bovine herpesvirus 1 can efficiently infect the human (SH-SY5Y) but not the mouse neuroblastoma cell line (Neuro-2A).

Prasanth Thunuguntla¹, Fouad S. El-mayet^{1,2}, and Clinton Jones^{1*}

¹: Oklahoma State University, Center for Veterinary Health Sciences, Department of Veterinary Pathobiology, Stillwater, OK 74078

²: Benha University, Faculty of Veterinary Medicine, Department of Virology, Moshtohor 13736, Kaliobyia, Egypt

*: Corresponding author

Keywords: Bovine herpesvirus 1 (BoHV-1), neuroblastoma cells, productive infection, Akt

Summary

Bovine herpesvirus 1 (BoHV-1) is a significant bovine pathogen that establishes a life-long latent infection in sensory neurons. Previous attempts to develop immortalized bovine neuronal cells were unsuccessful. Consequently, our understanding of the BoHV-1 latency-reactivation cycle has relied on studying complex virus-host interactions in calves. In this study, we tested whether BoHV-1 can infect human (SH-SY5Y) or mouse (Neuro-2A) neuroblastoma cells. We provide new evidence that BoHV-1 efficiently infects SH-SY5Y cells and yields virus titers approximately 100 fold less than bovine kidney cells. Conversely, virus titers from productively infected Neuro-2A cells were approximately 10,000 fold less than bovine kidney cells. Using a β -Gal expressing virus (gC-Blue), we demonstrate that infection of Neuro-2A cells (actively dividing or differentiated) does not result in efficient virus spread, unlike bovine kidney or SH-SY5Y cells. Additional studies demonstrated that lytic cycle viral gene expression (bICP4 and gE) was readily detected in SH-SY5Y cells: conversely bICP4 was not readily detected in productively infected Neuro-2A cells. Finally, infection of SH-SY5Y and bovine kidney cells, but not Neuro-2A cells, led to rapid activation of the Akt protein kinase. These studies suggest that the Neuro-2A cell line may be a novel cell culture model to identify factors that regulate BoHV-1 productive infection in neuronal cells.

Highlights:

- BoHV-1 efficiently infects human neuroblastoma cells (SH-SY5Y)
- BoHV-1 does not efficiently infect mouse neuroblastoma cells (Neuro-2A)
- A cellular protein kinase (Akt) is phosphorylated during early stages of infection

Bovine herpesvirus 1 (BoHV-1) is one of several viruses that is a co-factor during the development of a poly-microbial disease commonly referred to as bovine respiratory disease complex (BRDC), reviewed by (Jones and Chowdhury, 2007). BRDC is generally regarded as the most important disease in the cattle industry. Acute infection of cattle with BoHV-1 typically leads to upper respiratory tract disease, conjunctivitis, erosion of mucosal surfaces, and transient immune suppression (Hodgson et al., 2005; Jones and Chowdhury, 2010). *Mannheimia haemolytica* (MH) is a gram-negative bacterium that persists within the upper respiratory tract of healthy ruminants and is also associated with BRDC (Highlander, 2000, et al., 2001; Zecchinon L. et al., 2005). The commensal relationship between cattle and MH can be disrupted following stress or viral infection, and then MH becomes an important organism responsible for bronchopneumonia. BoHV-1 enhances interactions between the MH leukotoxin and peripheral blood mononuclear cells, including neutrophils, leading to acute inflammation in the lung and cell death (Leite et al., 2004; Rivera-Rivas et al., 2009). Calves co-infected with BoHV-1 and MH can also lead to life-threatening pneumonia (Yates et al., 1983). A BoHV-1 cellular entry receptor, the poliovirus receptor related 1 (PVRL1), was recently identified as a BRDC susceptibility gene for Holstein calves (Neibergs et al., 2014), underscoring the importance of BoHV-1 in BRDC.

Following acute infection, trigeminal ganglia (TG) are a primary site for life-long latency (Jones et al., 2011, 2013). Increased corticosteroid levels, due to food and water deprivation during shipping of cattle, weaning, and/or dramatic weather changes increase the incidence of BoHV-1 reactivation from latency, reviewed by (Jones, 2013, 2014). The synthetic corticosteroid dexamethasone (DEX), which mimics the effects of stress, stimulates productive infection (Kook

et al., 2015) and consistently initiates BoHV-1 reactivation from latency (Inman et al., 2002; Jones, 1998, 2003, 2006; Jones et al., 2000; Rock et al., 1992; Sheffy and Davies, 1972; Shimeld et al., 1990).

Although BoHV-1 has a strict tropism for cattle and other ungulates (Jones and Chowdhury, 2007), BoHV-1 can replicate in certain human cancer cells suggesting BoHV-1 has the potential to be an oncolytic virus (Cuddington and Mossman, 2014, 2015; Rodrigues et al., 2009). Since BoHV-1 is a neurotropic herpesvirus that establishes latency in sensory neurons, identifying neuroblastoma cells that support viral replication may provide a novel model to study virus-host interactions. Consequently, we tested whether BoHV-1 could grow in human (SH-SY5Y) or murine (Neuro-2A) neuroblastoma cells. These cell lines were chosen because they can be readily: 1) grown in culture, 2) differentiated into neuron-like cells, and 3) transfected (Shipley et al., 2016; Sinani et al., 2013, 2014; Tremblay et al., 2010). For initial studies, we utilized the gC-Blue virus, which contains the lacZ gene downstream of the gC promoter. This allows one to assess productive infection by counting β -Gal⁺ (beta-galactosidase positive) cells. The gC-Blue virus grows to similar titers as wt BoHV-1 in bovine cells and the number of β -Gal⁺ cells directly correlates with plaque formation (Geiser and Jones, 2002; Geiser et al., 2003; Inman et al., 2001; Meyer et al., 2007). Following infection of SH-SY5Y cells, we readily detected β -Gal⁺ cells by 8 hours after infection (Figure 1A). By 24 hours after infection, the SH-SY5Y monolayer was disrupted and most cells were floating, which was similar to bovine kidney cells (CRIB; data not shown).

In contrast to SH-SY5Y cells, we did not readily detect β -Gal⁺ cells at 8 hours after infection in Neuro-2A cells (data not shown). We used a MOI of 5 for infection studies with Neuro-2A cells because there were few β -Gal⁺ cells at 24 hours after infection when a MOI of 1 was used (data not shown). However, we readily detected β -Gal⁺ cells at 24 and 48 hours after infection when using a MOI of 5 (Figure 1B). Although fewer cells were observed at 48 hours after infection, the number of β -Gal⁺ cells had not increased dramatically suggesting virus spread or viral replication was not efficient. When Neuro-2A cells are differentiated by serum withdrawal as previously described (Sinani et al., 2013; Sinani and Jones, 2011), they typically differentiate into dopamine neurons that have “sprouted” long neurites (Tremblay et al., 2010) (Figure 1C and D). Following differentiation, we readily detected β -Gal⁺ cells at 24 and 48 hours after infection, but not in uninfected cells (Figure 1C). We did not observe clusters of β -Gal⁺ differentiated cells confirming viral replication was not efficient, cell-cell spread did not readily occur, or viral entry receptors were expressed at low levels in these cells. Higher magnification of differentiated Neuro-2A cells that were infected revealed lacZ expression was detected in the cell body and neurites (Figure 1D, denoted by blue arrows). As judged by lack of lacZ staining, uninfected differentiated cells were also present in the same field, (Figure 1D, black arrows).

Viral titers were subsequently measured following infection of Neuro-2A and SH-SY5Y cells. As a comparison, CRIB cells were infected. At 24 and 48 hours after infection, we consistently obtained yields approaching 1×10^8 plaque forming units (PFU) in CRIB cells (Figure 2). In contrast to CRIB cells, virus yields were approximately 100 fold less in SH-SY5Y cells and approximately 10,000 fold less in Neuro-2A cells infected with BoHV-1. We also

consistently detected 3-4 lower levels of infectious virus in Neuro-2A cells at 48 hours after infection. We attribute this, in part, to previous findings that Neuro-2A cells undergo apoptosis when growth factors are limiting or when cold-shocked: conversely this happen less frequently in fibroblasts (Li et al., 2010; Shen and Jones, 2008). Although BoHV-1 does not grow efficiently in Neuro-2A cells, infection may also contribute to cell death because infected cultures were not as healthy 48 hours after sub-culturing. These studies correlated well with the findings in Figure 1 because it was clear that Neuro-2A cells were infected but virus did not spread throughout the monolayer.

Viral protein expression was subsequently compared in CRIB, SH-SY5Y, and Neuro-2A cells to determine whether there was a correlation between viral protein expression and virus yield. As expected, the viral regulatory protein (bICP4) and a viral glycoprotein (gE) were readily detected in CRIB cells as well as SH-SY5Y cells (Figure 3A). The gE protein migrates as several bands on a Western blot because it is glycosylated at its amino terminus (Whitbeck et al., 1996). In contrast, bICP4 was not readily detected at 24 or 48 hours after infection, unless the Western Blot was over-exposed (Figure 3B). This finding provided further evidence that BoHV-1 does not efficiently replicate in Neuro-2A cells. In contrast to the results obtained with BoHV-1, herpes simplex virus 1 (HSV-1), another alpha-herpesvirinae subfamily member, replicates and spreads with similar efficiency in SH-SY5Y and Neuro-2A cells (data not shown).

We have observed a dramatic increase in phosphorylation of the cellular protein kinase Akt at serine 473 after infection following infection of primary bovine cells (Zhu et al., 2016), which correlates with Akt activation, reviewed in (Manning et al., 2007; Scheid and Woodgett,

2003). Akt is constitutively expressed in most cell lines, but activation requires phosphorylation at threonine 308 and serine 473 by two cellular protein kinases; PDK1 (phosphoinositide-dependent kinase) and mTORC2 [mTOR (mammalian target of rapamycin) complex 2]. Akt has many functions, including interfering with cell death (Franke et al., 1997). Infection of CRIB cells with BoHV-1 led to a dramatic increase of phosphorylated Akt at serine 473 within 4 hours after infection (Figure 4A). The levels of p-Akt at time 0 and in uninfected cultures 24 hours later were nearly undetectable, which was dramatically different compared to infected cultures. Within 4 hours after infection of SH-SY5Y or CRIB cells, p-Akt levels increased more than 20 fold relative to total Akt levels (Figure 4B and D). Following infection of Neuro-2A cells there was also an increase of p-Akt levels relative to Akt total protein (Figure 4C and D). The induction of p-Akt levels in Neuro-2A cells was not as dramatic as in CRIB and SH-SY5Y cells because p-Akt was consistently detected in uninfected Neuro-2A cells (Figure 4C, lane 0). Conversely, p-Akt was difficult to detect in uninfected CRIB or SH-SY5Y cells (Figure 4 A and B respectively). In summary, there was a strong correlation between bICP4 and gE protein expression and virus yield of CRIB or SH-SY5Y and Neuro-2A cells. These studies also provided evidence that efficient BoHV-1 infection correlated with increased Akt phosphorylation at serine 473.

In conclusion, this study demonstrated that BoHV-1 readily infected the human neuroblastoma cell line, SH-SY5Y. Although BoHV-1 was able to infect Neuro-2A cells (differentiated or rapidly dividing), virus replication and spread was not efficient. It is currently not clear whether BoHV-1 cellular receptors are only expressed in a subset of Neuro-2A cells, the virus does not spread efficiently in Neuro-2A cells, or crucial factors that regulate viral gene

expression are limiting in Neuro-2A cells. Consequently, Neuro-2A cells have the potential to be a useful model to identify cellular factors that regulate BoHV-1 replication.

ACKNOWLEDGEMENTS

This research was supported by a grant from the USDA-NIFA Competitive Grants Program (13-01041), funds derived from the Sitlington Endowment, and support from the Oklahoma Center for Respiratory and Infectious Diseases (National Institutes of Health Centers for Biomedical Research Excellence Grant # P20GM103648).

REFERENCES

- Cuddington, B.P. and K.L. Mossman, 2014. Permissiveness of human cancer cells to oncolytic bovine herpesvirus 1 is mediated in part by KRAS activity. *J Virol* 88, 6885-6895.
- Cuddington, B.P. and K.L. Mossman, 2015. Oncolytic bovine herpesvirus type 1 as a broad spectrum cancer therapeutic. *Current Opinions in Virology* 13, 11-16.
- Franke, T.F., D.R. Kaplan, and L.C. Cantley, 1997. PI3K: downstream AKTion blocks apoptosis. *Cell* 88, 435-437.
- Geiser, V., and C Jones, 2003. Stimulation of bovine herpesvirus 1 productive infection by the adenovirus E1A gene and a cell cycle regulatory gene, E2F-4. *J Gen Virol* 84, 929-938.
- Geiser, V., M. Inman, Y. Zhang, and C. Jones, 2002. The latency related (LR) gene of bovine herpes virus 1 (BHV-1) can inhibit the ability of bICP0 to activate productive infection. *J Gen Virol* 83, 2965-2971.
- Highlander, S.K., 2001. Molecular genetic analysis of virulence in *Mannheimia (Pasteurella) haemolytica*. *Front Biosci*, D1128-1150.
- Highlander, S.K., N. D. Fedorova, D. M. Dusek, R. Panciera, L. E. Alvarez, and C. Renhart, 2000. Inactivation of *Pasteurella (Mannheimia) haemolytica* leukotoxin causes partial attenuation of virulence in a calf challenge model *Infect Immun* 68, 3916-3922.
- Hodgson, P.D., P. Aich, A. Manuja, K. Hokamp, F.M. Roche, F.S.L. Brinkman, A. Potter, L.A. Babiuk, and P.J. Griebel, 2005. Effect of stress on viral-bacterial synergy in bovine respiratory disease: novel mechanisms to regulate inflammation. *Comparative and Functional Genomics*. 6, 244-250.

Inman, M., L. Lovato, A. Doster, and C. Jones, 2001. A mutation in the latency-related gene of bovine herpesvirus 1 leads to impaired ocular shedding in acutely infected calves. *J Virol* 75, 8507-8515.

Inman, M., L. Lovato, A. Doster, and C. Jones, 2002. A mutation in the latency related gene of bovine herpesvirus 1 interferes with the latency-reactivation cycle of latency in calves. *J Virol* 76, 6771-6779.

Jones, C., 1998. Alphaherpesvirus latency: its role in disease and survival of the virus in nature. *Adv Virus Res* 51, 81-133.

Jones, C., 2003. Herpes simplex virus type 1 and bovine herpesvirus 1 latency. *Clin Micro Rev* 16, 79-95.

Jones, C., 2013. Bovine herpes virus 1 (BHV-1) and herpes simplex virus type 1 (HSV-1) promote survival of latently infected sensory neurons, in part by inhibiting apoptosis. *J of Cell Death* 6, 1-16.

Jones, C., 2014. Reactivation from latency by alpha-herpesvirinae subfamily members: a stressful stimulation. *Current Topics in Virology* 12, 99-118.

Jones, C., L.F. da Silva, and D. Sinani, 2011. Regulation of the latency-reactivation cycle by products encoded by the bovine herpesvirus 1 (BHV-1) latency-related gene. *J Neurovirol* 17, 535-545.

Jones, C., T.J. Newby, T. Holt, A. Doster, M. Stone, J. Ciacci-Zanella, C.J. Webster, and M.W. Jackwood, 2000. Analysis of latency in cattle after inoculation with a temperature sensitive mutant of bovine herpesvirus 1 (RLB106). *Vaccine* 18, 3185-3195.

Jones, C., V. Geiser, G. Henderson, Y. Jiang, F. Meyer, S. Perez, and Y. Zhang., 2006. Functional analysis of bovine herpesvirus 1 (BHV-1) genes expressed during latency. *Vet Micro* 113, 199-210.

Jones, C. and S. Chowdhury, 2007. A review of the biology of bovine herpesvirus type 1 (BHV-1), its role as a cofactor in the bovine respiratory disease complex, and development of improved vaccines. *Adv in Anim Health* 8, 187-205.

Jones, C. and S. Chowdhur, 2010. Bovine herpesvirus type 1 (BHV-1) is an important cofactor in the bovine respiratory disease complex, in: Broderon, B. and V.L. Cooper (Ed.), *Veterinary Clinics of North America, Food Animal Practice, Bovine Respiratory Disease*. Elsevier, New York, NY, pp. 303-321.

Kook, I., C. Henley, F. Meyer, F. Hoffmann, and C. Jones, 2015. Bovine herpesvirus 1 productive infection and the immediate early transcription unit 1 are stimulated by the synthetic corticosteroid dexamethasone. *Virology* 484, 377-385.

Leite, F., C. Kuckleburg, D. Atapattu, R. Schulz, and C.J. Czuprynski, 2004. BHV-1 infection and inflammatory cytokines amplify the interaction between *Mannheimia haemolytica* leukotoxin with bovine peripheral blood mononuclear cells in vitro. *Veterinary Immunology and Immunopathology* 99, 193-202.

Li, S., D. Carpenter, C. Hsiang, S.L. Wechsler, and C. Jones, 2010. The herpes simplex virus type 1 latency-associated transcript (LAT) locus inhibits apoptosis and promotes neurite sprouting in neuroblastoma cells following serum starvation by maintaining active AKT (protein kinase B). *J Gen Virol* 91, 858-866.

Manning, B.D. and L.C. Cantley, 2007. AKT/PKB: navigating down stream. *Cell* 129, 1261-1274.

Meyer, F., S. Perez, V. Geiser, M. Sintek, M. Inman, and C. Jones 2007. A protein encoded by the bovine herpes virus 1 (BHV-1) latency related gene interacts with specific cellular regulatory proteins, including the CCAAT enhancer binding protein alpha (C/EBP- α). *J Virol* 81, 59-67.

Neibergs, H.L., C.M. Seabury, A.J. Wojtowicz, Z. Wang, E. Scraggs, J.N. Kiser, M. Neupane, J.E. Womack, A. Van Eenennaam, G.R. Hagevortm T.W. Lehenbauer, S. Aly, J. Davis, J.F. Taylor, and the Bovine Respiratory Disease Complex Coordinated Agricultural Research Team, 2014. Susceptibility loci revealed for bovine respiratory disease complex in pre-weaned holstein calves. *BMC Genomics* 15, 1-19.

Rivera-Rivas, J.J., D. Kisiela, and C.J. Czuprynski., 2009. Bovine herpesvirus type 1 infection of bovine bronchial epithelial cells increases neutrophil adhesion and activation. *Veterinary Immunol. And Immunopathol.* 131, 167-176.

Rock, D., J. Lokensgard, T. Lewis, and G. Kutish, 1992. Characterization of dexamethasone-induced reactivation of latent bovine herpesvirus 1. *J Virol* 66, 2484-2490.

Rodrigues, R., B. Cuddington, and K. Mossman, 2009. Bovine herpesvirus type 1 as a novel oncolytic virus. *Cancer Gene Therapy*, 1-12.

Scheid, M.P. and J.R. Woodgett, 2003. Unraveling the activation of protein kinase B/Akt. *FEBS Letters* 546, 108-112.

Sheffy, B.E. and D.H. Davies, 1972. Reactivation of a bovine herpesvirus after corticosteroid treatment. *Proc Soc Exp Biol Med* 140, 974-976.

Shen, W. and C. Jones, 2008. Open reading frame 2, encoded by the latency-related gene of bovine herpesvirus 1, has antiapoptotic activity in transiently transfected neuroblastoma cells. *J Virol* 82, 10940-10945.

Shimeld, C., T.J. Hill, W.A. Blyth, and D.L. Easty, 1990. Reactivation of latent infection and induction of recurrent herpetic eye disease in mice. *J Gen Virol* 71, 397-404.

Shipley, M., C.A. Mangold, and M.L. Szpara, 2016. Differentiation of the SH-SY5Y human neuroblastoma cell line. *JOVE* 108, e53193.

Sinani, D., L. Frizzo da Silva, and C. Jones, 2013. A bovine herpesvirus 1 protein expressed in latently infected neurons (ORF2) promotes neurite sprouting in the presence of activated Notch1 or Notch3. *J Virol*, 1183-1192.

Sinani, D., Y. Liu, and C. Jones, 2014. Analysis of a bovine herpesvirus 1 protein encoded by an alternatively spliced latency related (LR) RNA that is abundantly expressed in latently infected neurons. *Virology* 464-465, 244-252.

Sinani, D. and C. Jones, 2011. Localization of sequences in a protein encoded by the latency related gene of bovine herpesvirus 1 (ORF2) that inhibits apoptosis and interferes with Notch1 mediated trans-activation of the bICP0 promoter. *J Virol* 85, 12124-12133.

Tremblay, R., M. Sikorska, J.K. Sandhu, P. Lanthier, M. Ribocco-Lutkiewicz, and M. Bani-Yaghoub, 2010. Differentiation of mouse Neuro-2A cells into dopamine neurons. *J of Neuroscience Methods* 186, 60-67.

Whitbeck, J.C., A.C. Knapp, L.W. Enquist, W.C. Lawrence, and L.J. Bello, 1996. Synthesis, processing, and oligomerization of bovine herpesvirus 1 gE and gI membrane protein. *J Virol* 70, 7878-7884.

Yates, W.D., L. A. Babiuk, and K. W. Jericho, 1983. Viral-bacterial pneumonia in calves: duration of the interaction between bovine herpesvirus 1 and *Pasteurella haemolytica*. *Can J Comp Med* 47, 257-264.

Zecchinon L., T.F., and D. Desmecht 2005. How *Mannheimia haemolytica* defeats host defense through a kiss of death mechanism. *Vet Res* 36, 133-156.

Zhu, L., J. Thompson, F. Ma, J. Eudy, and C. Jones, 2016. Effects of the synthetic corticosteroid dexamethasone on early events during bovine herpesvirus 1 productive infection. Virology Submitted.

FIGURE LEGENDS

Figure 1. Infection of neuroblastoma cells with gC-Blue strain of BoHV-1.

Panel A: SH-SY5Y cells (ATCC: CRL-2266) were infected with the gC-Blue strain of BoHV-1 using a multiplicity of infection (MOI) of 1 PFU/cell. The gC-Blue strain of BoHV-1 (gCblue) contains the Lac Z gene in place of the viral gC gene, and was obtained from S. Chowdhury (Baton Rouge, LA). The virus grows to similar titers as the wild type parental virus and expresses the β -Gal gene as a late gene. At 8 hours after infection, cells were fixed and stained for Lac Z expression. As a control, uninfected cells were stained.

Panel B: Neuro-2A cells (ATCC: CCL-131) were infected with the gC-Blue strain of BoHV-1 using a MOI of 5 PFU/cell. At 24 or 48 hours after infection, cells were fixed and stained for Lac Z expression. As a control, uninfected cells were stained.

Panel C: Neuro-2A cells were differentiated by serum withdrawal (0.5% fetal calf serum for 72 hours) and then infected with the gC-Blue strain of BoHV-1 using a MOI of 5 PFU/cell. At 24 or 48 hours after infection, cells were fixed and stained for Lac Z expression.

Panel D: Higher magnification of differentiated Neuro-2A cells that were infected with BoHV-1 for 24 hours. Blue arrows denote β -Gal⁺ cells that exhibit staining in the cell body as well as in neurites. Black arrows denote differentiated Neuro-2A cells that were uninfected, as judged

by lack of β -Gal staining. For panels A-D, the respective cultures were washed in PBS, fixed (2% formaldehyde and 0.2% glutaraldehyde in phosphate-buffered saline [PBS]) for 5 minutes, and then stained with (1% Blue-Gal, 5 mM potassium ferricyanide, and 0.5 M MgCl_2 in PBS). Cultures were observed microscopically and these pictures are representative of more than 5 independent studies.

Figure 2. Measurement of viral infection in neuroblastoma cells. Neuro-2A or SH-SY5Y cells (1×10^6 cells / 100 cm^2 plates) were infected with BoHV-1 (1 PFU/cell) for 24 or 48 hours. Infected cells were scraped from dishes. Cells and medium (10 ml) were collected and total virus prepared by three cycle of freeze-thawing (-80°C to 37°C). Cell debris was removed by centrifugation for 10 minutes ($10,000 \times \text{G}$) and the supernatant was collected for measuring virus titers in CRIB (established bovine kidney) cells. The results presented are the amount of virus per ml from the total clarified lysate (10 ml): these values are the average of three independent studies. The Cooper strain of BoHV-1 was used for all of these studies and was obtained from the National Veterinary Services Laboratory, Animal and Plant Health Inspection Services, Ames, IA. BoHV-1 stocks were prepared in CRIB cells.

Figure 3. Detection of viral proteins after infection. Panel A: Confluent CRIB or SH-SY5Y cells in 100 mm dishes were infected with BoHV-1 at a MOI of 1 PFU/cell for the designated times after infection (hours). Neuro-2A cells were infected with a MOI of 5 PFU/cell for 0, 24 or 48 hours. Cells were lysed with RIPA buffer, proteins separated by SDS-PAGE, and

Western blot analysis performed using a peptide specific bICP4 antibody (position of bICP4 is denoted by black circle) or gE specific antibody (gE-specific bands denoted by bracket) that was obtained Dr. Lynn Enquist (Princeton). As a loading control, the levels of tubulin were examined. For each lane, 50 ug protein was loaded.

Panel B: Neuro-2A samples in Panel A were exposed longer to visualize bICP4 in infected cells. The position of molecular weight markers is shown on the right side of the respective gels. These results are representative of three independent studies.

Figure 4. BoHV-1 infection stimulated Akt phosphorylation. Confluent CRIB (Panel A) or SH-SY5Y cells (Panel B) in 100 mm dishes were infected with BoHV-1 at a MOI of 1 PFU/cell for the designated times after infection (hours). Neuro-2A cells (Panel C) were infected at a MOI of 5 PFU/cell. Cells were lysed with RIPA buffer, proteins separated by SDS-PAGE, and Western blot analysis performed to detect expression of phosphorylated AKT (Cell Signaling Technology; catalogue# 9271) and total Akt (Cell Signaling Technology; catalogue# 9272), or tubulin. For each lane, 50 ug protein was loaded. Lanes denoted 16C or 24C were mock-infected cells that were cultured in media. Levels of p-AKT phosphorylation were measured by comparing the levels in p-AKT bands to that in total Akt bands at the same time point. Since we were unable to readily detect p-Akt in uninfected CRIB and SH-SY5Y cells, we likely underestimated the effects of infection on increasing p-Akt levels. The results shown are the mean of three independent experiments and error bars denote the variability. The position of molecular weight markers is shown on the right side of the respective gels.

Figure 1

Figure 2

Figure 3

Figure 4