KOR. JOUR. MICROBIOL February, 1993 p. 79~84 Vol. 31, No. 1

Calcium Response of CHSE Cells Following Infection with Infectious Pancreatic Necrosis Virus (IPNV)

Kang, Kyung-Hee[1], Kee-Soon Park[1], Joo-Hyun Yoon[1] and Chan-Hee Lee[1,2]*

[1]Department of Microbiology, College of Natural Science, Chungbuk National University, Cheongju 360-763, and [2]Research Center for Molecular Microbiology, Seoul National University, Seoul 151-742, Korea

Infection of Chinook Salmon Embryo (CHSE) cells with IPNV resulted in a significant decrease in intracellular free calcium concentration ($[Ca^{2+}]i$) compared to mock-infected cells. The degree of the decrease in $[Ca^{2+}]i$ was dependent on the amount of input virus, and treatment of IPNV-infected CHSE cells with metabolic inhibitors such as cycloheximide or cordycepin partially reversed the decrease in $[Ca^{2+}]i$ in IPNV-infected cells. Inactivation of IPNV with UV also abolished IPNV-induced decrease in $[Ca^{2+}]i$. These data suggest an active role of IPNV in the decrease of $[Ca^{2+}]i$ in the infected CHSE cells. The importance of the decrease in $[Ca^{2+}]i$ could be supported by the finding that the production of IPNV plaques increased in the cells treated with verapamil, a calcium influx blocker, and by lowering the concentration of extracellular calcium. Decreased production of IPNV plaques was observed by elevating the extracellular calcium. Thus, it is suggested that IPNV induced a decrease in $[Ca^{2+}]i$ and the decrease in $[Ca^{2+}]i$ may play an important role in efficient replication of IPNV.

KEY WORDS □ **IPNV, calcium, CHSE cells**

Infection of cells with viruses resulted in marked changes in intracellular microenvironment and these changes may be important in the replication of the viruses. In some cases, these changes involve the alteration of the intracellular levels of signal transduction elements. For example, increased breakdown of intracellular inositol lipids (30), increased intracellular uptake of calcium followed by increase in $[Ca^{2+}]i$ (20), and increased cAMP and cGMP (2) were observed following infection of human fibroblast cells with human cytomegalovirus (HCMV). Those cellular changes seem to be required for HCMV replication since altering the normal cellular changes by drugs such as papaverine inhibited HCMV replication. Involvement of signal transduction elements in virus replication or gene expression has also been reported in other viruses (4, 8, 15, 31).

Infectious pancreatic necrosis virus (IPNV) is an important pathogen for salmonoid fishes. The IPNV genome contains two segments of double-stranded RNA, characteristic of family Birnaviridae (9, 10) and codes for at least four different polypeptides. Based on the serological cross-reactivity of virion proteins, a number of different strains have been identified worldwide including Ab, Sp, VR-299, Jasper, and WB. Recently Park *et al.* (23) isolated a new serotype of IPNV from rainbow trout (*Salmo gairderi*) in Korea. The new serotype, DRT, has been identified to be serologically and molecular biologically distinguishable from three reference strains of IPNV, Ab, Sp, and VR-299 (19, 23). Although some progress has been noted in immunological and molecular biological studies of IPNV, relatively little is known for virus-cell interaction in *in vitro* cell culture. Furthermore, very little work has been focused on the cellular changes following IPNV infection. As a first step in understanding the cellular changes following IPNV infection, this study aimed to study the calcium response in IPNV-DRT-infected CHSE cells.

MATERIALS AND METHODS

Virus and cell culture

IPNV strain DRT originally isolated in Korea (23) and chinook salmon embryo (CHSE) cells were used in this study. CHSE cells were cultured in EMEM (Eagle's minimum essential medium, Earle's salt) supplemented with 10% newborn bovine serum (NBS) and 0.075% $NaHCO_3$ at 18°C.

Plaque assay

The infectious titer of IPNV-DRT was deter-

mined by plaque assay. Confluent monolayer of CHSE cells grown in 35 mm tissue culture dishes was inoculated with diluted virus stock or sample and overlayed with EMEM supplemented with 2% NBS, 0.25% agarose, 100 U/ml penicillin, 100. μg /ml streptomycin, and 1 μg/ml Fungizone. Cells were fixed with 10% formalin two days after virus infection and stained with 0.03% methylene blue.

Plaque reduction assay

Effect of drugs or modulation of calcium concentration was investigated by plaque reduction assay. Confluent monolayer of CHSE cells grown in 35 mm tissue culture dishes was infected with IPNV-DRT with inoculum of 50~100 plaques/dish. After 1 hr adsorption at 18°C, overlay medium containing drugs or varying calcium concentration was added to the infected cultures. Cell monolayer was fixed 2 days after infection, stained with methylene blue and the number of plaques was counted.

RNA synthesis

Confluent monolayer of CHSE cells was infected with IPNV-DRT at MOI of approximately 3 pfu (plaque forming unit)/cell. At selected times after virus infection, ^{3}H-uridine (Amersham, Arlington Heights, IL, U.S.A., specific activity=25 Ci/mmol) was added to the infected cultures at a final concentration of 5 μCi/ml. Infected cultures were frozen at −20°C, thawed and digested overnight with 0.06 ml protease (1%), 0.03 ml EDTA (0.02 M) and 0.03 ml sodium sarcosine (2 %) per ml of medium. A small aliquot (100 μl) of the digest was spotted onto Whatman No.3 filter paper and subjected to three cycles of trichloroaetic acid (TCA, 5%) precipitation. Filter papers were dried and the radioactivity was counted using scintillation counter.

Measurement of intracellular free calcium concentration

Fluorescent indicator of calcium, Fura-2/AM (Molecular Probes, Eugene, OR, U.S.A.) was used to measure the concentration of intracellular free calcium. CHSE cells grown in tissue culture flasks were infected or mock-infected with IPNV-DRT. At selected times after virus infection, cells were washed twice with PBS and loaded with Fura-2 /AM (10 μM in loading buffer: 10 mM Hepes, pH 7.4, 130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 10 mM sodium pyruvate, 0.02% pluronic-F127) for 1 hr at 18°C. Cells were harvested by trypsinization and centrifugation and washed twice with loading buffer. Fluorescence was measured using fluorescence spectrophotometer (Hitachi F-3000). Excitation wavelength and emission wavelength were 340 nm and 500 nm, respectively.

RESULTS AND DISCUSSION

IPNV-DRT growth cycle in CHSE cells

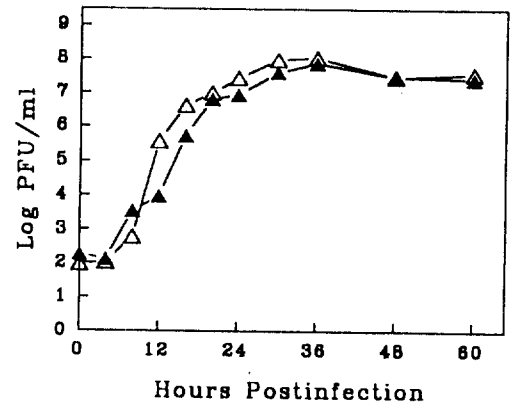

Fig. 1. *One-step growth cycle of IPNV-DRT in CHSE cells.*
Confluent monolayer of CHSE cells grown in tissue culture flasks was infected with IPNV-DRT at MOI of 3 pfu/cell. Infectious virus titers from extracellular fluids (▲) or total cultures (△) were determined at the times indicated by plaque assay.

In order to understand the kinetics of production of infectious IPNV-DRT, confluent monolayer of CHSE cells grown in tissue culture flasks was infected with IPNV at MOI (multiplicity of infection) of 3 or 10 pfu/cell and the infectious titers of IPNV in extracellular fluid or total cultures were determined by plaque assay. The data presented in Fig. 1 indicates that infectious virus particles began to be produced at 8 hr postinfection (p.i.) and accumulated in the cells until 16 hr p.i., after which release of infectious virus particles rapidly increased. Maximum titer of IPNV, cell-free or total, was obtained at 36 hr p.i. and then there was slight decrease in virus titer. Similar result was observed with MOI of 10 pfu/cell, except that maximum titer was obtained at 24 hr p.i. (data not shown).

The RNA synthesis in IPNV-DRT-infected CHSE cells was studied by determining the incorporation of ^{3}H-uridine into acid insoluble precipitates. RNA synthesis began as early as 2 hr p.i. and reached maximum at 6 hr p.i. (Fig. 2). Thereafter, the level of RNA synthesis slightly decreased until 18 hr p.i. followed by rapid decrease. It is not definitely known at this time whether the observed radioactivity was due to the syntheis of IPNV RNA or cellular RNA or both. It seems reasonable, however, to attribute the observed synthesis of RNA in IPNV-infected CHSE cells to IPNV RNA synthesis since most RNA viruses effectively suppress cellular RNA synthesis (13).

Changes in intracellular free calcium concentration following IPNV-DRT infection

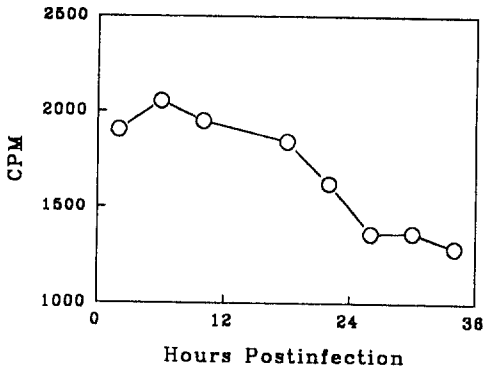

Fig. 2. *RNA synthesis in CHSE cells infected with IPNV-DRT.*
CHSE cells grown in 35 mm tissue culture dishes were infected with IPNV-DRT at MOI of 3 pfu/cell. Radioactive ^{3}H-uridine (specific activity=25 Ci/mmol, final concentration=5 μCi/ml) was added to the cultures every 4 hrs and the amount of radioactivity in TCA-precipitate was counted.

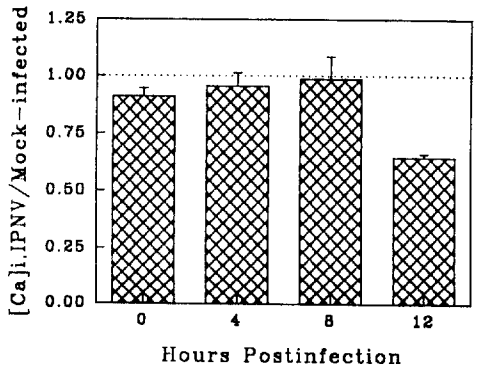

Fig. 3. *The calcium response in CHSE cells to IPNV-DRT infection.*
CHSE cells grown in 25 cm^2 tissue culture flasks were infected with IPNV-DRT at MOI of 3 pfu/cell. At selected times after infection, cells were loaded with Fura-2/AM (10 μM) and the fluorescence was measured.

The concentration of intracellular free calcium was determined by using Fura-2/AM. Confluent monolayer of CHSE cells was infected or mock-infected with IPNV-DRT at MOI of 3 pfu/cell, loaded with Fura-2/AM at 4 hr time interval for 12 hrs, and the fluorescence was measured. Since the purpose of this study was to investigate the effect of IPNV-DRT infection on the changes of $[Ca^{2+}]i$, the ratio of fluorescence in IPNV-infected cells relative to the fluorescence in mock-infected cells was obtained. As shown in Fig. 3,

Table 1. *The effect of different MOI on intracellular free calcium concentration in IPNV-DRT-infected CHSE cells*

MOI	Fluorescence	Percent of control	Percent change
15	27.9± 1.20	79.3	−20.7
3	22.0± 0.91	62.5	−37.5
0.6	28.9± 0.52	82.1	−17.9
0.12	35.1± 0.87	99.7	− 0.3
0.024	34.6± 2.44	98.3	− 1.7
0	35.2± 1.48	100	−

Table 2. *The effect of UV and metabolic inhibitors on intracellular free calcium concentration in IPNV-DRT-infected CHSE cells*

Treatment	Fluorescence	Percent of control	p value*
Exp#1			
Mock	25.5± 1.25	100	
IPNV	20.3± 1.27	80	
IPNV+UV	31.3± 1.27	123	<0.05
Exp#2			
Mock	38.1± 0.76	100	
IPNV	21.7± 0.69	57.0	
IPNV+cycloheximide	27.2± 0.47	71.4	<0.05
IPNV+cordycepin	32.9± 2.31	86.4	<0.05

*compared to IPNV-DRT-infected cultures

the relative ratio of fluorescence was not changed significantly until 8 hr p.i. but at 12 hr p.i. there was significant reduction in the relative ratio of fluorescence ($p<0.01$).

The data also showed that at all times investigated $[Ca^{2+}]i$ in IPNV-infected cells was lower than that in mock-infected cells. Therefore, IPNV-DRT infection resulted in decrease in $[Ca^{2+}]i$. The effect of the amount of input virus on the decrease in $[Ca^{2+}]i$ was investigated in order to understand whether the decrease in $[Ca^{2+}]i$ was due to IPNV infection. As MOI increased from 0.024 to 3 pfu/cell, there was a gradual enhancement in the level of IPNV-induced decrease in $[Ca^{2+}]i$ (Table 1), indicating a relationship between the amount of virus infected per cell and the level of decrease in $[Ca^{2+}]i$. Higher MOI of 15 pfu/cell resulted in less profound effect on $[Ca^{2+}]i$ than MOI of 3 pfu/cell and this may be explained by direct killing effect due to too much virus infected per cell.

The decrease in $[Ca^{2+}]i$ following IPNV-DRT infection appears to require infectious virus since UV-inactivated IPNV did not reduce the $[Ca^{2+}]i$ (Table 2). Metabolic inhibitors such as cycloheximide (inhibitor of protein synthesis) or cor-

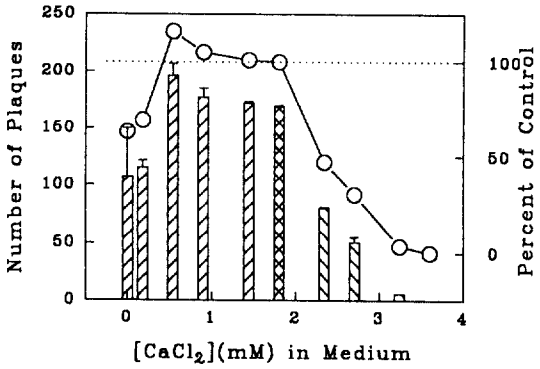

Fig. 4. *Effect of varying extracellular calcium concentration on production of plaques on CHSE cells.*
Overlay medium containing various concentration of $CaCl_2$ was added to CHSE cells infected with IPNV-DRT (approximately 150 pfu/dish). Cells were fixed 2 days after infection and the number of plaques was counted. Bars, number of plaques; line, percent of control (relative to 1.8 mM).

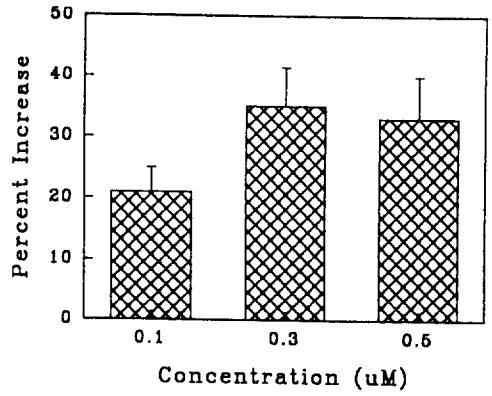

Fig. 5. *Effect of verapamil on plaque formation of IPNV-DRT.*
Confluent monolayer of CHSE cells grown in 35 mm dishes was infected with IPNV-DRT (approximately 70 pfu/cell) and overlayed with medium containing various concentration of verapamil. The number of plaques was counted at 2 days after infection.

dycepin (3'-deoxyadenosine; inhibitor of RNA synthesis) partially, but significantly ($p<0.05$) blocked the IPNV-induced decrease in $[Ca^{2+}]i$ (Table 2). These data suggest that the observed decrease in $[Ca^{2+}]i$ following IPNV infection was due to expression of gene functions. It is not clear whether the decrease in $[Ca^{2+}]i$ was due to viral or cellular functions since cycloheximide or cordycepin could block either IPNV or cellular metabolic activities or both. However, it seems possible that viral gene function(s), at least in part, is involved in the decrease in $[Ca^{2+}]i$ since the level of decrease in $[Ca^{2+}]i$ was positively related to the amount of input virus as shown in Table 1.

Our data suggesting a decrease in $[Ca^{2+}]i$ following IPNV-DRT infection of CHSE cells sharply contrasts to those obtained by Nokta *et al.* (20) who demonstrated a rapid influx of extracellular calcium into the human cytomegalovirus (HCMV)-infected human fibroblast cells followed by gradual but steady increase in $[Ca^{2+}]i$. The difference might be due to the different intrinsic ability of the two viruses to activate quiescent cells. HCMV is well known to activate quiescent or resting cells (1). HCMV induces cellular DNA synthesis (28, 29), enhanced expression of proto-oncogenes fos, *myc* and *jun* (6, 7), and alterations in cellular protein synthesis (14) including suppression of fibronectin gene expression (17, 22) and induction of cellular heat shock protein synthesis (26). All these changes occurred following infection of HCMV are to a certain degree related to the phenomena collectively known as cell activation (3). Since activation of a quiescent cell usually includes alteration of signal transduction elements such as calcium, inositol lipids and cyclic nucleotides (5, 25), it is not surprising that HCMV infection of permissive human cells induces changes in $[Ca^{2+}]i$ toward cell activation, i.e., increase in $[Ca^{2+}]i$ (2).

The mechanism for the decrease in $[Ca^{2+}]i$ in IPNV-DRT-infected CHSE cells is not understood at this moment. It could be possible, however, that IPNV-DRT capsid protein might act as a calcium-binding protein. The capsid proteins of some icosahedral viruses contain calcium-binding sites and are involved in holding the capsid together and probably serve as sensors to tell the virus when it has entered the submicromolar calcium environment of a cell interior (11, 12, 16, 24). The $[Ca^{2+}]i$ in IPNV-DRT infected CHSE cells decreased slightly during the first 8 hr after virus infection and this may be due to binding of intracellular free calcium to IPNV-DRT virion proteins. As suggested from the data presented in Fig. 2, viral RNA synthesis reaches maximum at 6 hr p.i.. Capsid proteins might be synthesized in a large amount after RNA synthesis. Intracellular virus particles are accumulated until 16 hr p.i. (data from Fig. 1). Thus, the observed significant decrease in $[Ca^{2+}]i$ at 12 hr p.i. might be explained by binding of intracellular free calcium to newly synthesized viral capsid proteins. Another possibility is that IPNV-DRT infection somehow stimulates the activity of Ca^{2+}-ATPase on the membrane of intracellular calcium sequestering organelles such as endoplasmic

reticulum or mitochondria. Similarly possible is stimulation of the activities of Na^+/Ca^{2+} antiport or Ca^{2+} ATPase on plasma membrane.

Although little is known about the effect of IPNV on cellular gene functions, it seems reasonable to admit that IPNV, like most RNA viruses (13), suppresses cellular gene functions. Most RNA viruses, including Reovirus whose genome is also double-stranded RNA, are not well known to induce cell activation. Thus, it is possible to hypothesize that IPNV infection would not result in cell activation as HCMV does.

Effect of calcium on IPNV multiplication

The data shown above suggest that calcium is important in IPNV-DRT replication. If decreased $[Ca^{2+}]i$ is important in IPNV-DRT replication, then reversing the decrease of calcium concentration might interfere with IPNV-DRT replication. This possibility was tested by plaque reduction assay in the presence of various concentration of calcium in extracellular medium (in the form of $CaCl_2$, 1.8 mM in EMEM). The data summarized in Fig. 4 clearly indicates that the number of IPNV-DRT plaques was significantly ($p<0.05$) reduced by enhancing the concentration of calcium in extracellular medium from 1.8 mM (1x) to 2.34 (1.3x), 2.7 (1.5x), 3.24 (1.8x) and 3.6 mM (2x). The number of IPNV-DRT plaques in the presence of higher calcium concentration was reduced to 47%, 30%, 3.6% and 0%, respectively. On the other hand, slightly more (1 to 16%) plaques were produced when the calcium concentration of extracellular medium was lowered to 1.44 (0.8x), 0.9 (0.5x) and 0.54 mM (0.3x). When the concentration of calcium in extracellular medium was lowered to 0.18 mM (0.1x) or 0 mM, production of viral plaques was decreased significantly ($p<0.05$), suggesting that extremely low concentration of calcium may be negative for IPNV-DRT replication. Thus, IPNV-DRT multiplication was dependent on the calcium concentration in extracellular medium and there was dose-dependent decrease of IPNV-DRT multiplication as the concentration of calcium increased from 0.54 mM to 3.6 mM. Similar conclusion could be drawn from the experimental results shown in Fig. 5. Verapamil, a well known calcium-influx blocker (27 for review), significantly ($p<0.05$) increased the number of IPNV-DRT plaques.

Very little work has been reported on the role of calcium on virus replication. As far as we know, our data showing reverse relationship of calcium concentration and IPNV-DRT multiplication contrast to the data obtained for influenza virus (21) and herpes simplex virus (18). In the latter two viruses, verapamil inhibited the replication of the viruses.

In conclusion, infection of CHSE cells with IPNV-DRT results in a significant decrease in $[Ca^{2+}]i$ measured at 12 hr p.i. which requires infectious virus and gene function(s).

ACKNOWLEDGEMENTS

This work has been supported by a KOSEF Research Grant for SRC (Research Center for Molecular Microbiology, Seoul National University).

REFERENCES

1. **Albrecht, T., I. Boldogh, M. Fons, C.H. Lee, S. AbuBakar, J.M. Russell, and W.W. Au,** 1989. Cell-activation responses to cytomegalovirus infection relationship to the phasing of CMV replication and to the induction of cellular damage. *Subcell. Biochem.* **15**, 157-202.
2. **Albrecht, T., M.P. Fons, I. Boldogh, S. AbuBakar, C.Z. Deng, and D. Millinoff,** 1991. Metabolic and cellular effects of human cytomegalovirus infection. *Transplant. Proc.* 23 suppl **3**, 48-55.
3. **Alkon, D.L. and H. Rasmussen,** 1988. A spatial-temporal model of cell activation. *Science* **239**, 998-1004.
4. **Barker, K., A. Aderem, and H. Hanafusa,** 1989. Modulation of arachidonic acid metabolism by Rous sarcoma virus. *J. Virol.* **63**, 2929-2935.
5. **Berridge, M.J.,** 1987. Inositol lipids in cell proliferation. *Biochim. Biophys. Acta* **907**, 33-45.
6. **Boldogh, I., S. AbuBakar, and T. Albrecht,** 1990. Activation of proto-oncogenes: an immediate early event in human cytomegalovirus infection. *Science* **247**, 561-564.
7. **Boldogh, I., S. AbuBakar, C.Z. Deng, and T. Albrecht,** 1991. Transcriptional activation of cellular oncogenes *fos, jun,* and *myc* by human cytomegalovirus. *J. Virol.* **65**, 1568-1571.
8. **Davies, A.H., R.J.A. Grand, F.J. Evans, and A.B. Rickinson,** 1991. Induction of Epstein-Barr virus lytic cycle by tumor-promoting and non-tumor-promoting phorbol esters requires active protein kinase. C. *J. Virol.* **65**, 6838-6844.
9. **Dobos, P.,** 1976. Size and structure of the genome of infectious pancreatic necrosis virus. *Nucl. Acid Res.* **3**, 1903-1924.
10. **Dobos, P., B.J. Hill, H. Ross, D.T.C. Kells, H. Becht, and D. Teninges,** 1979. Biophysical and biochemical characterization of five animal viruses with bi-segmented double-stranded RNA genomes. *J. Virol.* **32**, 593-605.
11. **Durham, A.C.H., D.A. Hendry, and M.B. Von Wechmar,** 1977. Does calcium ion binding control plant virus disassembly? *Virology* **77**, 524-533.
12. **Eisenmann, G., and O. Alvarez,** 1990. Structure and selectivity of Ca-binding sites in proteins: the 5-fold site in an icosahedral virus, p. 283-299. *In* D. Pasu, and F. Bronner (eds.), Calcium transport and intracellular calcium homeostasis. NATO ASI Series H48, Springer-Verlag, Berlin,

Heidelberg, New York.
13. **Fernandez-Tomas, C.,** 1987. Virus-induced suppression of host transcription, p. 21-58. *In* L. Carrasco (ed.), Mechanisms of viral toxicity in animal cells. CRC Press, Boca Raton.
14. **Grundy, J.E.,** 1991. Alterations of cellular proteins in human cytomegalovirus infection: potential for disease pathogenesis. *Transplant. Proc.* 23 suppl 3, 38-42.
15. **Hofer, F., B. Berger, M. Gruenberger, H. Machat, R. Dernick, U. Tessmer, E. Kuechler, and D. Blaas,** 1992. Shedding of a rhinovirus minor group binding protein: evidence for a Ca^{2+}-dependent process. *J. Gen. Virol.* **73**, 627-632.
16. **Hull, R.,** 1978. The stabilization of the particles of turnip rosette virus. III. Divalent cations. *Virology* **89**, 418.
17. **Ihara, S., S. Saito, and Y. Watanabe,** 1982. Suppression of fibronectin synthesis by an early function(s) of human cytomegalovirus. *J. Gen. Virol.* **59**, 409-413.
18. **Lee, C.H.,** 1990. Effect of calcium on herpes simplex virus type 1 replication. *J. Kor. Soc. Virol.* **20**, 115-122.
19. **Lee, J.J., J.W. Park, G. Jeong, and Y.C. Hah.,** 1989. A new serotype confirmed by partial physical mapping of cDNA clones from the infectious pancreatic necrosis virus (IPNV) isolated in Korea. *Kor. J. Microbiol.* **27**, 231-236.
20. **Nokta, M., D. Eaton, O.S. Steinsland, and T. Albrecht,** 1987. Ca^{2+} responses in cytomegalovirus-infected fibroblasts of human origin. *Virology* **157**, 259-267.
21. **Nugent, K.M., and J.D. Shanley,** 1984. Verapamil inhibits influenza A virus replication. *Arch. Virol.* **81**, 163-170.
22. **Pande, H., T. Terramani, T. Tressel, M.A. Churchill, G.G. Hawkins, and J.A. Zaia,** 1990. Altered expression of fibronectin gene in cells infected with human cytomegalovirus. *J. Virol.* **64**, 1366-1369.
23. **Park, J.W., J.J. Lee, G. Jeong, and Y.C. Hah,** 1989. Characterization of infectious pancreatic necrosis virus (IPNV) isolated from pan-cultured rainbow trout in Korea. *Kor. J. Microbiol.* **27**, 225-230.
24. **Rossmann, M.G., C. Abad-Zapatero, M.R.N. Murthy, L. Liljas, T.A. Jones, and B. Stanbergm,** 1983. Structural comparisons of some small spherical viruses. *J. Mol. Biol.* **165**, 711-736.
25. **Rozengurt, E.,** 1986. Early signals in the mitogenic response. *Science* **234**, 161-166.
26. **Santomenna, L.D. and A.M. Colberg-Poley,** 1990. Induction of cellular hsp70 expression by human cytomegalovirus. *J. Virol.* **64**, 2033-2040.
27. **Singh, B.N., G. Ellrodt, and C.T. Peter,** 1978. Verapamil: a review of its pharmacological properties and therapeutic use. *Drugs* **15**, 169-197.
28. **St. Jeor, S.C. and R. Hutt,** 1977. Cell DNA synthesis as a function in the replication of human cytomegalovirus. *J. Gen. Virol.* **37**, 65-73.
29. **St. Jeor, S.C., T.B. Albrecht, F.D. Funk, and F. Rapp,** 1974. Stimulation of cellular DNA synthesis by human cytomegalovirus. *J. Virol.* **13**, 353-362.
30. **Valyi-Nagy, T., Z. Bandi, I. Boldogh, and T. Albrecht,** 1988. Hydrolysis of inositol lipids: an early signal of human cytomegalovirus infection. *Arch. Virol.* **101**, 199-207.
31. **Vainionpaa, R., T. Hyypia, and K.E.O. Ackerman,** 1991. Early signal transduction in measles virus-infected lymphocytes is unaltered, but second messengers activate virus replication. *J. Virol.* **65**, 6747-6748.

(Received November 10, 1992)
(Accepted December 2, 1992)

초 록: 전염성 췌장 괴저 바이러스 감염에 따른 CHSE 세포의 칼슘 반응

강경희[1] · 박기순[1] · 윤주현[1] · 이찬희[1,2]* ([1]충북대학교 자연과학대학 미생물학과, [2]서울대학교 분자미생물학연구센터)

Chinook salmon embryo(CHSE) 세포가 전염성 췌장 괴저 바이러스(IPNV)에 감염되면 감염되지 않은 세포에 비해 세포내 유리 칼슘의 농도가 감소한다. 칼슘 농도 감소의 정도는 넣어준 감염성 바이러스 양에 의존하며, IPNV에 감염된 세포를 cycloheximide나 cordycepin 같은 대사억제제를 처리하였을 때 부분적으로 회복되었다. 또한 IPNV를 자외선으로 불활성화시키면 IPNV에 의해 유도되는 유리 칼슘 농도의 감소는 보이지 않았다. 이러한 결과는 감염된 CHSE 세포에서 세포내 유리 칼슘 농도를 감소시키는 과정에 IPNV가 직접적으로 관여하고 있음을 보여주고 있다. IPNV 증식에 있어서의 세포내 유리 칼슘 농도 감소의 중요성은 칼슘 유입 억제제인 verapamil을 처리하거나, 세포외 칼슘의 농도를 감소시킨 배지에서 배양한 세포에서 IPNV의 plaque 생성 능력이 증가했다는 사실로도 뒷받침될 수 있다. IPNV plaque 수의 감소는 세포외 칼슘 농도를 증가시켜 주었을 때 관찰되었다. 따라서 이러한 결과들은 IPNV가 세포내 유리 칼슘 농도의 감소를 유도하며, 이러한 세포내 유리 칼슘 농도의 감소는 아마도 IPNV가 세포내에서 효과적으로 증식하는데 중요한 역할을 할 것이라는 사실을 제시해 준다.