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Scrapie Strain Infection in Vitro Induces Changes in Neuronal Cells

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Abstract

PC12 cells, in the presence of nerve growth factor (NGF), support replication of the mouse-derived scrapie strains 139A and ME7, with the former yielding 100–1000-fold higher levels of infectivity. Infectivity remained cell-associated and cells did not show any gross morphological alterations, although changes were observed by electron microscopy in the form of an increased number of lipid droplets in 139A-infected cultures. Analysis of phospholipid metabolism in 139A infected cells indicated that scrapie replication did not change the inositol phosphate levels, but did stimulate phosphoinositide synthesis. Replication was not detected in PC12 cells infected with either the hamster-derived 263K or rat-derived 139R scrapie strains.

Since scrapie-infected cultures did not exhibit cell death or any gross changes, any scrapie-induced effects would probably be manifested in nonvital cellular functions. When compared to controls, infection with the 139A scrapie strain resulted in decreased activity of the cholinergic pathway-related enzymes, as well as the GABA synthetic pathway; however, the adrenergic pathway was unaffected by scrapie infection. The effects of the 139A scrapie strain on the cholinergic system appeared to be dose-dependent and were first detected prior to the detection of scrapie agent replication in these cells. No neurotransmitter-related enzymatic changes were detected in 263K- or 139R-infected PC12 cells. The enzymatic changes observed in ME7-infected PC12 cells and in Chandler agent-infected mouse neuroblastoma cells suggest that the significant changes in neurotransmitter levels in cultures exhibiting low infectivity titers must involve factors other than, but not excluding, replication of the agent. The role of additional factors is also suggested in studies of protein kinase C activity in 139A- and 139R-infected PC12 cells. These studies emphasize the value of the PC12 cell model system in examining the scrapie strain–host cell interaction and, in addition, support the concept of variation among scrapie strains.

Index Entries: Scrapie strains; PC12 cells; neuronal; neurotransmitters; lipid droplets; phospholipids.

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Introduction

Scrapie, a central nervous system (CNS) disease affecting sheep and goats, is a prototype of the transmissible subacute spongiform encephalopathies (TSSE). This group includes a number of diseases in humans and animals: kuru, Gerstmann-Sträussler Scheinker disease (GSS), Creutzfeldt-Jakob disease (CJD), transmissible mink encephalopathy, chronic wasting disease of mule deer and elk, and bovine spongiform encephalopathy. Scrapie has been experimentally transmitted to mice and hamsters, which serve as useful model systems to study the disease. Disease-specific structures termed scrapie-associated fibrils (SAF) have been found in tissues containing scrapie infectivity *(1)*. The major component of these structures is a protease-resistant protein, PrP^{SC} *(2)*, which is the modified form of a host-coded glycoprotein, PrP^{C}. Although SAF, PrP, and infectivity are related, controversy exists concerning the nature of the scrapie agent. Three major hypotheses for the agent have been proposed: virus, prion, and virino *(3)*. Extensive work by Dickinson and others *(4–6)* has demonstrated the existence of scrapie strains in mice. These strains can be differentiated from each other based on incubation period, histopathological changes, and clinical characteristics in inbred strains of mice. In addition, Kimberlin and others have demonstrated the existence of hamster scrapie strains *(7)*. A consistent finding in scrapie pathology is that if scrapie-related factors (strain, dose, and origin of agent) and host factors (strain and sex of host, route of injection) are kept constant, the pathology and pathogenesis associated with the disease process are remarkably reproducible *(8)*.

The establishment of a tissue-culture system that would support scrapie agent replication might enhance our understanding of the nature of the agent, and would help in defining the mechanisms associated with neuropathogenesis of this and related diseases. There have been numerous attempts to establish scrapie replication in cell culture systems. These studies used cell cultures derived from infected brains *(9–11)* or in vitro infection of established cell lines with agent *(11–14)*. These systems yielded unimpressive infectivity titers, generally 1 LD_{50}/100–1000 cells. The poor levels of replication may be a consequence of several factors, including the cell types employed and the comparatively rapid rate of cell division relative to the intrinsically slow rate of scrapie agent replication.

The major source for scrapie infectivity is the brain, with the neurons presumably being the site of agent replication. An in vitro neuronal model, such as PC12 cells, would therefore be useful to study cell–agent interactions. PC12 cells are a cloned tumor cell line derived from a rat pheochromocytoma *(15)*. In the continued presence of low concentrations of nerve growth factor (NGF), these cells stop dividing and undergo morphological, physiological, and biochemical neuron-like differentiation *(15–19)*.

This article describes studies examining scrapie agent replication in vitro using PC12 cells as a neuronal model. The effects of agent replication on cellular functions and the ability to demonstrate the existence of scrapie strains in a cell culture model are also described.

Replication of Various Scrapie Strains in Vitro

The ability of scrapie strains to replicate in cell cultures may be dependent not only on the type of cell, but also on the growth kinetics of a cell. We first examined the ability of PC12 cells to support scrapie strain replication. Once that was established, we ascertained if the differentiated state of the cell influenced that ability. PC12 cells were maintained in an NGF-induced differentiated state on collagen-coated substrates for approx 3 wk. Cells were infected at a multiplicity of infection (m.o.i.) of 1 with diluted 139A scrapie brain homogenate. Bioassays of cells harvested at weekly intervals after infection indicated that the 139A scrapie strain was able to replicate in PC12 cells continually maintained in the presence of NGF *(20,21)*. End point titration assays indicated that the 139A scrapie strain could achieve infectivity titers of up to approx 4 LD_{50}/cell, but 1 LD_{50}/cell was more common *(21)*. Since the removal of NGF causes PC12 cells to dedifferentiate, we examined whether this altered cellular state would influence scrapie strain replication. Therefore, we infected NGF-treated PC12 cells at an m.o.i. of 1 and removed the NGF 1 wk after infection. The absence of NGF caused the cells to lose their neuronal properties over several weeks and resume cell division. Bioassays of these cells indicated that at 3 wk after the removal of NGF, there was no evidence of replication of the 139A scrapie strain in these dedifferentiated cells *(20,21)*. These results indicated that cells must be in the

NGF-induced neuronal state to support scrapie strain replication.

The ability and extent to which scrapie agents were able to replicate in NGF-treated PC12 cells were dependent on the scrapie strain used (22). In addition to the 139A strain, the ME7 strain was able to replicate in these cells; however, the yields of infectivity were extremely low: 0.01–0.001 LD_{50}/cell. Furthermore, a hamster-adapted scrapie strain, 263K, did not replicate in differentiated PC12 cells. Unexpectedly, the 139R rat-adapted scrapie strain also did not replicate in differentiated PC12 cells (which are of rat origin) during the entire course of the experiment (42 d), as demonstrated by negative bioassay results in both rats and mice.

Although the NGF-treated PC12 cells were able to support scrapie strain replication to relatively high titers, the cells did not exhibit any gross cytopathic effects. Furthermore, the infectivity present in the infected cultures was found to be cell-associated and was not found in the media.

Effects of Scrapie Strain on "Luxury Functions" of PC12 Cells

Neurotransmitter Synthesis

Since scrapie strain replication in PC12 cultures does not cause any gross cytopathic changes, we proposed that any scrapie agent induced effects would be related to nonvital or luxury functions in these cells. One of the major luxury functions associated with differentiated neuronal cells is neurotransmitter metabolism. NGF-treated PC12 cells infected with the 139A scrapie strain demonstrated altered neurotransmitter-related enzyme activities (Fig. 1) (23). Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities were assayed as markers for the cholinergic system, and tyrosine hydroxylase (TH) and dopa decarboxylase were the indicators used for the adrenergic pathway. In addition to the excitatory neurotransmitters, we also examined the effects of scrapie agent replication on the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), by assaying glutamic acid decarboxylase (GAD) activity.

In cultures exposed to a 1:500 dilution (m.o.i. of 1) of brain homogenate of the 139A scrapie strain, ChAT activity began to decrease by 21 d post-infection (p.i.). ChAT activity continued to decrease until the end of the experiment (42 d p.i.), at which time 84% of the activity had been lost. The decrease in ChAT activity closely paralleled the kinetics of 139A scrapie strain replication in NGF-treated PC12 cells. AChE activity also decreased following 139A

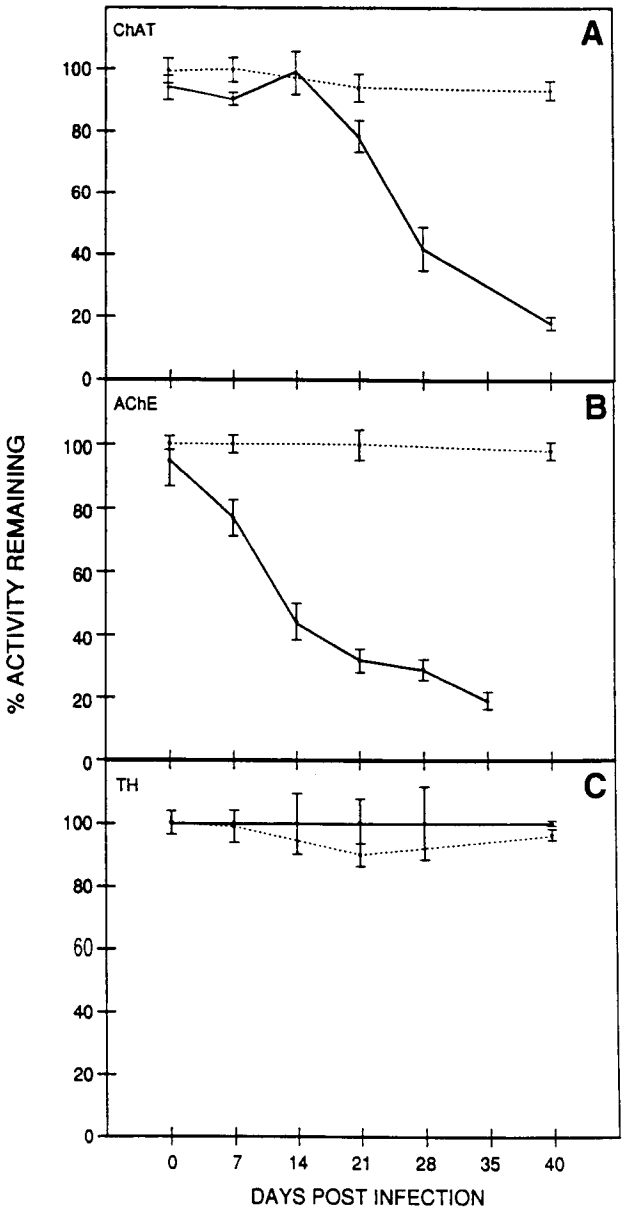

Fig. 1. Neurotransmitter-related enzyme activity in mock- (dashed line) and 139A scrapie-infected (solid line), NGF-treated PC12 cells. For each experiment the specific enzyme activity for ChAT **(A)**, AChE **(B)**, and TH **(C)** for each sample was calculated and the mean specific activity ± SEM was determined for the samples at each time-point. Each of these values was compared to the absolute activity from cultures exposed to normal mouse brain homogenate 5 h p.i. (0 d p.i.) and plotted as the percentage activity remaining.

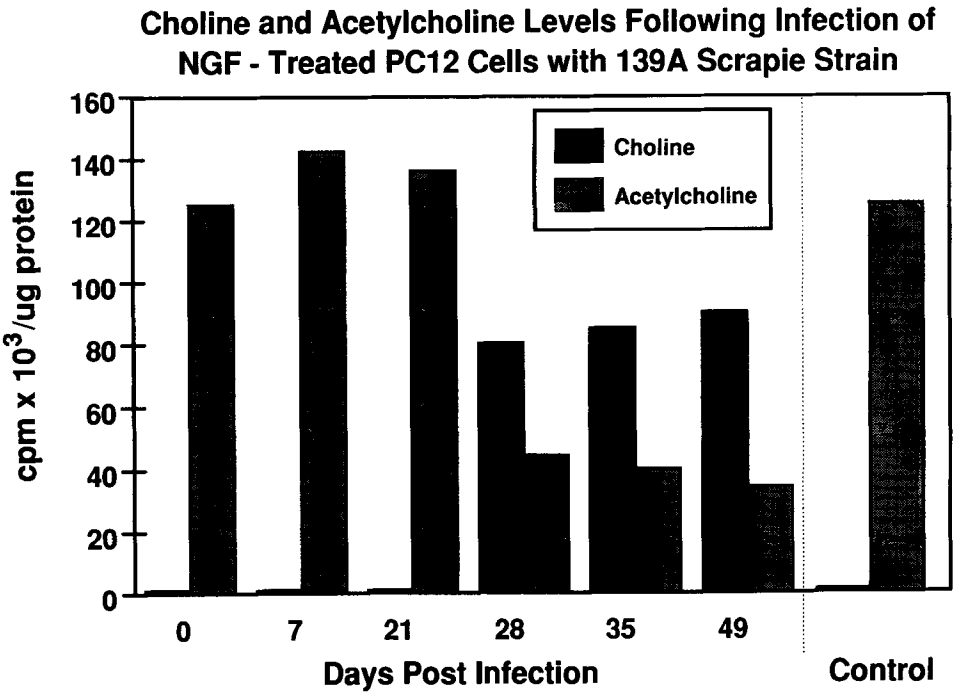

Fig. 2. Choline and ACh levels at various times after infection of NGF-treated PC12 cells with 139A scrapie strain. As a control, cells were exposed to normal mouse brain homogenate.

scrapie strain infection, but was detected earlier than agent replication could be measured. The decrease in AChE activity was first detected by 7 d p.i. and continued until the end of the experiment, at which time only 20% of the activity remained. The scrapie-induced changes in enzyme activity also occurred when partially purified PrP preparations were used as the inoculum indicating that these changes were scrapie-agent-specific and not owing to contaminating brain material (*23*). In addition to the changes in ChAT and AChE activities, our studies also showed that the levels of choline and acetylcholine (ACh) were affected by 139A scrapie strain replication (Fig. 2). Choline is converted into and stored as ACh in control cells and in cells tested early after infection with 139A. In cultures infected with the 139A scrapie strain, the rate of conversion of choline into ACh was significantly reduced by 28 d p.i. These results coincided with the reduction in enzyme activities and were consistent with the expected consequences of that reduction.

The adrenergic pathway was examined by assaying TH and dopa decarboxylase activities in 139A-scrapie-strain-infected NGF-treated PC12 cultures. The activities of both TH (Fig. 1) and dopa decarboxylase (data not shown) were similar in cells exposed to either normal or scrapie brain homogenate (*23*), and the activity levels in all groups of cultures remained relatively constant throughout the experiment.

Infection of NGF-treated PC12 cells with the 139A scrapie strain at an m.o.i. of 1 caused a dramatic decrease in GAD levels with activity reaching undetectable levels by 35 d p.i. (Fig. 3, top). In contrast, enzyme activities were not altered in cultures exposed to normal mouse brain homogenate.

All of the enzymatic activities affected by scrapie agent replication showed a dose–response relationship with regard to the concentration of inoculum used to infect the cultures (*23*). Cells were infected with various dilutions of 139A scrapie brain homogenate, harvested at 28 d p.i., and assayed for the activities of various enzymes. ChAT, AChE, (Fig. 4), and GAD (Fig. 3, bottom) activities decreased to a greater extent with increasing concentrations of inoculum. TH activity, as expected, was not altered at any dilution of brain homogenate used for infection (Fig. 4).

In addition to 139A, other scrapie strains were examined for their ability to alter enzymatic activity related to neurotransmitter metabolism (Fig. 5) (*22*). Infection of NGF-treated PC12 cells with the ME7 scrapie strain also caused a decrease in ChAT activity. This decrease was first detected at 28 d p.i.

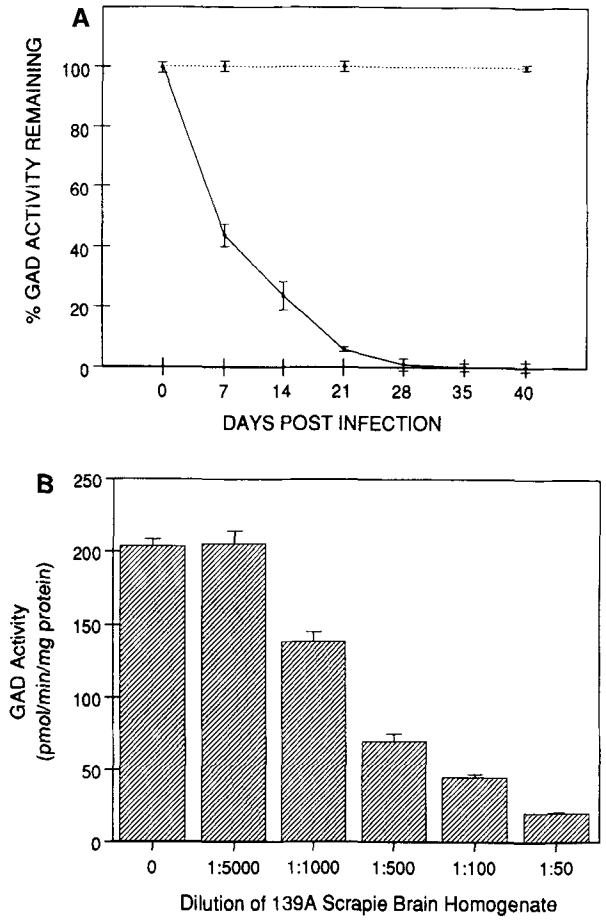

Fig. 3. **(Top)** GAD activity in NGF-treated PC12 cells following infection with the 139A scrapie strain. Following exposure to a 1:500 dilution of normal or scrapie brain homogenate, cells were harvested at the indicated times and assayed for GAD activity (pmol/min/mg protein). Values are expressed as mean ± SEM. **(Bottom)** Effect of the dose of inoculum on 139A scrapie strain-induced changes in GAD activity. PC12 cells were infected with various dilutions of 139A scrapie brain homogenate (in a diluent of 1:500 dilution of normal mouse brain homogenate). All cultures were harvested at 28 d p.i. and GAD activity was determined.

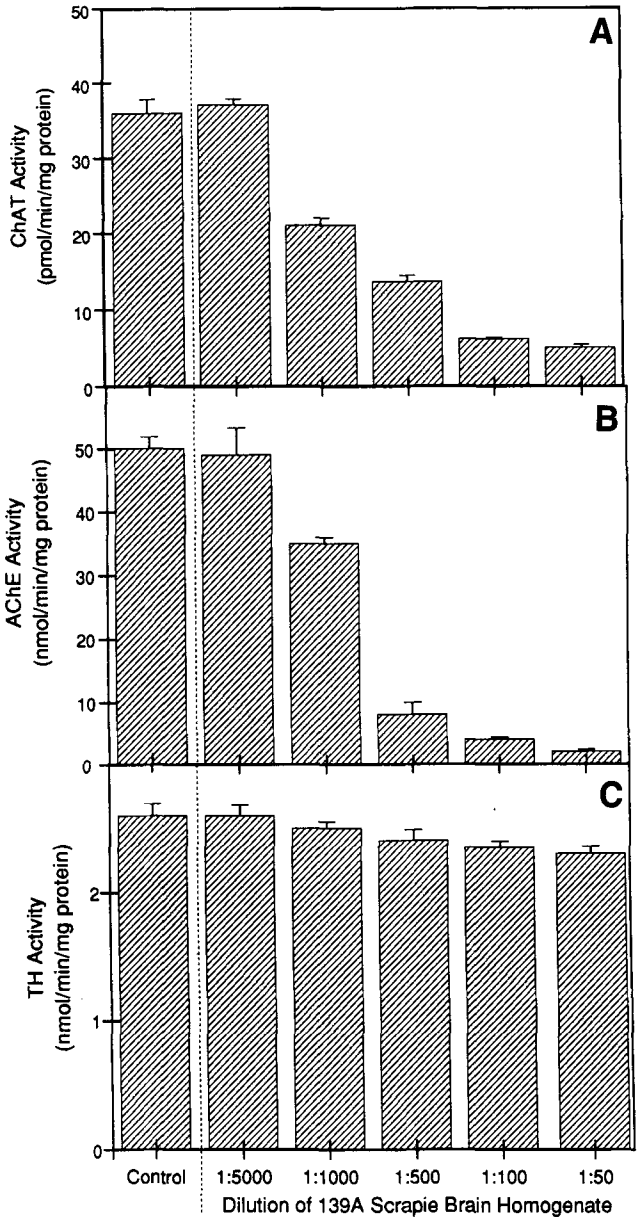

Fig. 4. Effect of the dose of inoculum on 139A scrapie strain-induced changes in neurotransmitter-related enzyme activities ([A] ChAT activity; [B] AChE activity; [C] TH activity). Cultures of differentiated PC12 cells were infected with various dilutions of 139A scrapie brain homogenate. All cultures were harvested at 28 d p.i., and the specific activities of the various enzymes were determined. The control sample represents the enzyme activity 28 d postexposure to a 1:500 dilution of normal mouse brain homogenate. All values are expressed as the mean specific activity ± SEM.

and resulted in a 48% decrease in ChAT activity by 42 d p.i. (compared to the 84% decrease seen for 139A-infected cells). Since the increases in scrapie infectivity and the decreases in enzymatic activities in PC12 cells are correlated temporally, it is probable that the changes in enzyme levels are a function of effects induced during scrapie agent replication. However, in comparing 139A and ME7, the relatively small differences in the reduction of enzymatic activity did not reflect the greater differences in infectivity titers. The failure of the 263K and 139R scrapie strains to replicate in PC12 cells was correlated with the finding that neither caused any changes in ChAT activity. TH activity was also

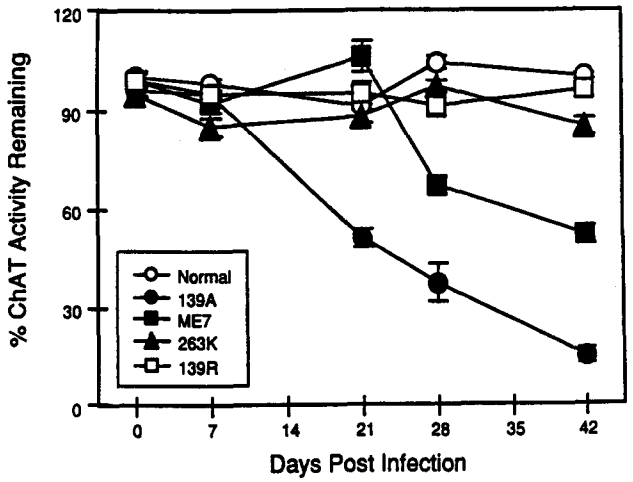

Fig. 5. ChAT activity in NGF-treated PC12 cells following infection with various scrapie strains. Following exposure to a 1:500 dilution of normal or scrapie brain homogenate, cells were harvested at the indicated times and assayed for ChAT activity (pmol/min/mg protein). Values are expressed as mean ± SEM.

examined following ME7, 263K, and 139R infection of NGF-treated PC12 cells, and was found to be unaltered regardless of the scrapie strain used (data not shown) *(22)*.

Since persistent replication of scrapie agent has also been observed in mouse neuroblastoma cells, we determined whether scrapie-induced neurotransmitter changes could also be detected in these cultures. Mouse neuroblastoma cells persistently infected with the Chandler scrapie strain (cell line 325-B6) and control cells exposed to normal brain homogenate (cell line 321-16) were examined. The scrapie-infected mouse neuroblastoma cells have been well characterized *(13,24)*, and those clones that contain PrP27-30, as shown by Western blotting, also contain scrapie infectivity. Infectivity studies *(24)* have demonstrated titers of 0.01–0.001 LD_{50}/cell. In these cells, ChAT activity was decreased by 32%, and AChE activity by 45% as compared to controls. In contrast, TH activity in the scrapie-infected mouse neuroblastoma cells was similar to that seen in controls (Table 1) *(22)*.

Electron Microscopic Observation of Lipid Droplets

Although scrapie-infected PC12 cells show no morphological alterations by phase-contrast microscopy, we have recently noticed cytoplasmic changes using transmission electron microscopy (TEM). At 4 wk postinfection, 139A scrapie-strain-infected NGF-treated PC12 cells were fixed with 2% formaldehyde–1% glutaraldehyde in 0.1*M* sodium cacodylate buffer for 1 h at 4°C. After fixation, the cells were washed in buffer containing sucrose, dehydrated in a graded ethanol series to 90% ethanol, and embedded in Lowicryl. Examination of sections by TEM indicated the presence of a large number of lipid droplets in the cytoplasm of scrapie-infected cultures. Similar structures were also present, to a lesser degree, in uninfected PC12 cells. Immunocytochemical studies were performed on the infected cells at the EM level. Sections were cut, blocked with 3% BSA in PBS for 30–60 min, and incubated with polyclonal rabbit antisera (raised against either PrP preparations or β-amyloid precursor protein), followed by binding of colloidal gold particles for antibody detection. Specific binding of colloidal gold particles was evident at the edge of the lipid droplets. Quantitatively, the PrP antibody was bound to 64% of the lipid droplets at 4 wk after 139A infection. In contrast, the PrP antibody bound to only 6% of the lipid droplets from PC12 cells exposed to normal mouse brain. The antibody binding to the infected cultures was specific for PrP as evident from the control studies using an antibody to the amyloid precursor protein involved in amyloid formation in Alzheimer's disease or secondary antibody alone (Table 2). It is likely that the antibody is binding to PrP^{sc}, since PrP^{c} synthesis does not increase during scrapie infection *(25)*.

Phospholipid Metabolism

Hydrolysis of membrane phosphoinositides was first described in pancreatic slices exposed to acetylcholine *(26)* and later shown to occur in many other systems as a consequence of the activation of a variety of receptors *(27)*. The two classes of metabolites generated by this hydrolysis, diacylglycerols and inositol phosphates, have been proposed to play the role of intracellular messengers. Hydrophobic diacylglycerols are the physiological activators of protein kinase C (PKC) *(27)*, a major mediator of signal transduction, whereas inositol triphosphate triggers the release of calcium from intracellular stores *(28)*. Diacylglycerol and inositol triphosphate are generated as a result of the hydrolysis of phosphatidylinositol.

It has been established that PrP^{C} and PrP^{SC} are anchored to the cell membrane via a glycosylphosphatidylinositol anchor *(29)*. It has been suggested that glycolipid-anchored proteins influence enzymatic density, mobility, and release of proteins

Table 1
Neurotransmitter-Related Enzyme Activity
in Chandler Agent-Infected and Mock-Infected Mouse Neuroblastoma Cells

Treatment	ChAT, pmol/min/mg protein	AChE, pmol/min/mg protein	TH, nmol/min/mg protein
Mock-infected (321-16)	18.96 ± 1.51	9.96 ± 1.87	8.67 ± 1.88
Scrapie-infected (325-B 6)	12.85 ± 1.45	5.49 ± 1.21	8.94 ± 1.47

Table 2
Immunoelectron Microscopic Analysis of Lipid Droplets
in 139A-Infected PC12 Cells 4 Wk Posttreatment

	Immunocytochemistry[a]		
Treatment	PrP antibody	Beta-APP antibody	Secondary antibody only
139A	333[a]/520 (64%)	23/239 (10%)	10/239 (4%)
Normal mouse brain	26/434 (6%)	12/132 (9%)	6/159 (4%)

[a]Number of lipid droplets that contain at least 1 gold particle at the edge/total number of lipid droplets.

from the membrane, as well as the regulation of surface receptors and second-messenger systems.

Phospholipid metabolism was analyzed according to previously described methods *(30,31)*. Mock and 139A-infected NGF-treated PC12 cells were incubated in the presence of ^{3}H-inositol for 60 min. Either prior to or after incubation in the presence of 10 μ*M* bradykinin (bradykinin stimulates the incorporation of inositol into inositol phosphates and inhibits incorporation into phosphoinositides), the cells were harvested, treated with organic solvents to extract inositol-containing phospholipids, and analyzed by both thin-layer and anion exchange chromatography. The levels of inositol, inositol phosphates, glycosyl-phosphatidylinositol, and phosphoinositides were measured prior to and following the addition of bradykinin. At 4 wk p.i., the levels of free inositol, glycosyl-phosphatidylinositol, and inositol phosphates were not altered (Fig. 6). However, a scrapie-strain-induced increase of phosphoinositide levels was found in 139A-infected cultures. This increase (difference between cultures infected with scrapie and normal brain homogenates) was slight, but significant in untreated cultures and more pronounced in bradykinin treated cells (Fig. 6). Whether this is owing to stimulated synthesis or inhibition of breakdown is as yet unclear.

PKC activity in the infected PC12 cells was measured using the assay described by Yasuda et al. *(32)*. No change in PKC activity was detected before 7 wk p.i., the time when other scrapie-related alterations had been present for several weeks. A decrease in PKC activity was seen, however, 7 wk p.i. in both 139A- and 139R-exposed cultures (data not shown).

Discussion

There have been many attempts to establish a scrapie-infected tissue-culture cell line. In some reports, scrapie infectivity that was present initially decreased to undetectable levels as the cells were continually passaged *(11)*. Other reports demonstrated infectivity at all cell passages *(9,10,13,14)*, but the infectivity levels were extremely low. In some of these studies, cloned cell lines of scrapie-infected cells have been established *(14,24)*.

Differentiated PC12 cells were able to support the replication of two mouse scrapie strains, 139A and ME7, to different degrees. Although both scrapie strains replicated in this system, the yield of infectivity from 139A-infected cultures was greater than that from ME7-infected cultures by two to three orders of magnitude. Furthermore, the 139A-infected cells caused a more dramatic change in neurotransmitter enzymes compared to the ME7 infected cultures. The hamster scrapie strain, 263K, and the rat-adapted scrapie strain, 139R, did not replicate or cause neurotransmitter-related changes in PC12 cells. The inability of 263K to replicate may be related to the species barrier phenomenon that exists in vivo *(33,34)*. The species barrier would not play a role in the inability of the 139R strain to

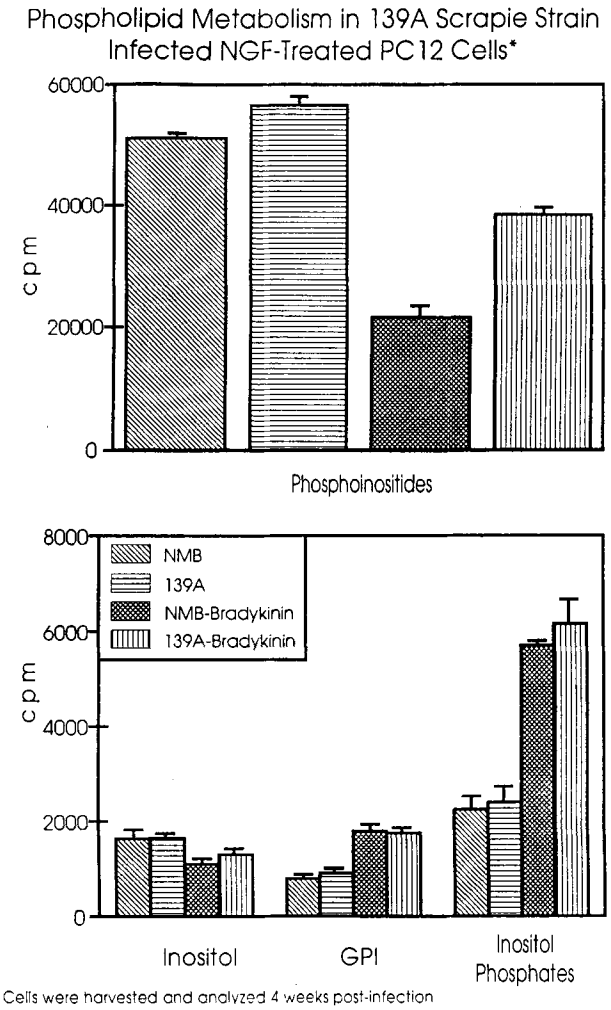

Fig. 6. NGF-treated PC12 cells were exposed to normal mouse brain (NMB) or 139A brain homogenate for 4 wk prior to analysis. Cells were incubated in inositol-free RPMI 1640 plus 5 μCi/mL ^{3}H-inositol. Following a 60-min labeling, the reaction was stopped with ice-cold TCA. Phosphoinositides, inositol phosphates, and glycerophosphatidylinositol (GPI) were analyzed using thin-layer chromatography followed by organic extraction previously described by Koreh and Monaco *(30)*.

replicate, since PC12 cells are of rat origin. It may be necessary to use a higher m.o.i. for the 139R scrapie strain. It is also possible that PC12 cells, which were derived from the New England Deaconess Hospital strain of rats, carry a gene that makes them susceptible to the 139A strain, but resistant to the 139R strain, which was passaged in outbred Lewis albino rats.

Butler et al. *(14)* has reported scrapie agent (Chandler strain) replication in mouse neuroblastoma cells when infected with an m.o.i. of 100, but not when an m.o.i. of 1 was used. Since an m.o.i. of 1 was used for infection of PC12 cells with the 139A and ME7 strains of scrapie, it suggests that PC12 cells can be infected with a greater efficiency than some other cell lines. It is not known whether scrapie strain replication in differentiated PC12 cells is a function of the specific cell type, the result of nondividing state of NGF-treated PC12 cells, or is a function of other NGF-related effects. It has been shown previously that NGF causes many cellular changes, including: stimulation of transcription *(35–37)*, an increase in PrP mRNA in cholinergic neurons *(38)*, and an increase in PrP expression in PC12 cells. Whether these or other NGF-related effects influence scrapie replication in these cells is uncertain.

Scrapie agent replication in NGF-treated PC12 cells caused altered activity of specific neurotransmitter-related enzymes. The changes were observed early after infection and prior to the earliest detection of infectivity in the PC12 cells. The dramatic changes seen in ChAT, AChE, and GAD activities at early times p.i. suggested that at the time of infection most, if not all, of the cells were infected, and their nonvital or luxury functions affected. Whether the decreased enzyme activities were the result of diminution in the specific activities of the enzymes or inhibition of new enzyme synthesis remains to be established.

The replication of the 139A scrapie strain in PC12 cells usually yields 1 infectious U/cell. This could be the result of a few cells producing many infectious units per cell or all cells producing low levels of infectivity. Results with the ME7-PC12 system and with the Chandler agent-infected mouse cell model demonstrate that although the yields of inactivity per cell were extremely low (0.01–0.001 infectious U/cell), a 30–50% reduction in ChAT activity and a 45% reduction in AChE were observed. There are two possible mechanisms that could lead to these findings. One mechanism is that many cells are infected, but few are producing agent at any given time; the group of cells that are infected but are not producing agent may still show changes in their luxury functions induced by the nonproductive infectious process going on within the cells. Supporting this concept are cell cloning studies by Race et al. *(24)*. In their studies of infected cultures of neuroblastoma cells, nearly every cell was infected even when infectivity yields were only 0.01–0.001 infectious U/cell. Another possible mechanism would involve release of a soluble substance by the few productively infected cells; this substance would then affect luxury functions of many of the uninfected cells within the culture.

The scrapie-specific changes in enzyme activities may be the result of a direct effect of the agent on neurotransmitter pathways or an indirect action on other cellular systems. For example, the scrapie agent could alter the action of NGF on PC12 cells. NGF causes a reversible increase in PC12 cell neurotransmitter metabolism *(15)*. Blocking the effects of NGF by binding to NGF receptors or interfering with NGF-related second-messenger systems could alter normal enzyme activity; however, scrapie agent infection does not cause dedifferentiation in the NGF-induced differentiated PC12 cells.

NGF has previously been shown to alter phospholipid metabolism in PC12 cells *(39)*. Phospholipid metabolism is involved in signal transduction and plays a role as a mediator in second-messenger systems. Infection of NGF-treated PC12 cells with the 139A scrapie strain altered phospholipid metabolism by causing an increase in phosphoinositide synthesis. Furthermore, preliminary studies have shown that the second-messenger-related enzyme, PKC, is decreased in both 139A- and 139R-infected NGF-treated PC12 cells. These results suggest that although scrapie strain replication is necessary for many of the effects seen in cells, there must be factors other than, or in addition to, replication that are responsible for some cellular changes. This latter point is evident by the finding that there are PKC changes in the 139R-PC12 system in which there is no scrapie agent replication.

Changes in scrapie-infected cells in vitro have previously been described at both the light microscopy *(40)* and EM *(41)* levels. These changes have included cytoplasmic vacuolization and cytoplasmic vesicles, which appear to be secondary lysosomes. The lipid droplets described in the present study may be similar to previously published ultrastructural changes or might represent a new observation. Whether these lipid droplets play a role in the infection process or are involved in the neurotransmitter or phospholipid changes is uncertain.

NGF has been reported to increase PrP^{C} mRNA levels in both PC12 cells *(42)* and in regions of developing hamster brain containing cholinergic neurons *(38)*. It can be speculated that PrP^{C} and PrP^{SC} are involved in regulation of neurotransmitter synthesis.

The ability of a virus to alter cellular luxury functions without affecting their vital functions was reported in murine neuroblastoma cells infected with lymphocytic choriomeningitis virus (LCMV) *(43)*. LCMV did not alter the cells morphology, but did reduce ACh levels by causing a decrease in both ChAT and AChE activities.

Scrapie-induced clinical disease and histopathological changes have been related to neuronal dysfunction, perhaps owing to specific alterations in luxury functions (i.e., neurotransmitter-related mechanisms) *(44–46)*. Studies of neurotransmitters in the brains of animals injected with scrapie and normal material have not yielded consistent findings, which could be a function of one of the following factors:

1. Different host strain–scrapie strain combinations used;
2. Different brain regions examined; and
3. Alterations in only a small population of cells that are masked by the great number of unaffected cells.

The use of a tissue-culture system can alleviate these problems and generate a well-defined cell population to study scrapie strain–host cell interations. With regard to other neurodegenerative diseases, the PC12 cell culture model may also be useful to examine mechanisms associated with cellular dysfunction leading to neuropathology and clinical disease.

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