Preservation of Catecholamine Uptake and Release in Herpes Simplex Virus Type 1-infected PC12 Cells

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SUMMARY

The PC12 cell line, which is derived from a rat pheochromocytoma and possesses a number of 'differentiated' neuronal properties, was used to characterize the effect of herpes simplex virus type 1 (HSV-1) infection on the uptake and release of catecholamines. Both the uptake of [3H]norepinephrine and the content and K+-induced release of endogenous catecholamines were remarkably preserved during the course of productive infection. HSV-1 infection is thus selective in its effects on the host cell and certain specialized functional properties may be retained in the face of otherwise profound metabolic alterations.

Herpes simplex virus type 1 (HSV-1) infection of neurons plays a central role both in the production of human disease and in the 'life cycle' of the virus in the human community. Thus, in HSV-1 encephalitis, a severe infection of the central nervous system accompanied by high mortality and morbidity, neurons are a major target of productive infection (Esiri, 1982), whereas neurons of the peripheral nervous system serve as the principal reservoirs of latent HSV, allowing periodic reactivation and transmission of the virus (Stevens, 1975). In an attempt to improve understanding of how neurons might be altered during the course of productive, and perhaps latent, infection, we have used the PC12 cell line as a cell culture model of infection. This cell line was cloned from a rat pheochromocytoma (Greene & Tischler, 1976) and exhibits a number of neuronal properties, including the capacity to synthesize, store, release and take up catecholamines (Greene & Rein, 1977a, b; Ritchie, 1979). On exposure to low concentrations of nerve growth factor (NGF), PC12 cells cease cell division and undergo further morphological and physiological differentiation with extension of neurites and development of an excitable membrane (Greene & Tischler, 1976; Rudy et al., 1982). We report here the effect of HSV-1 infection on the uptake, content and release of catecholamines in PC12 cells.

HSV-1 infection of PC12 cells has been previously characterized in our laboratory (Tsukamoto & Price, 1982; Rubenstein & Price, 1983). For the present experiments, PC12 cells were maintained at 37 °C in 93% air-7% CO2 atmosphere for 3 weeks in 35 mm plastic tissue-culture dishes (approx. 1.5 x 105 cells/dish) coated with air-dried rat-tail collagen (Bornstein, 1958) in RPMI medium supplemented with 100 ng/ml of 2.5S NGF (Mobley et al., 1976), antibiotics, 2 mM-glutamine and 2% horse serum. Under these conditions, PC12 cells cease cell division and undergo further morphological differentiation with extension of a plexus of neurites (Greene & Tischler, 1976). The NGF-treated cells were infected with a multiplicity of 10 p.f.u./cell of the F strain of HSV-1 prepared on rabbit kidney (RK) cells. The inoculum for studies using mock infection was similarly prepared from uninfected RK cells. As in previous studies, HSV-1 infection under these conditions resulted in production of viral progeny as well as in morphological cytopathology that was detectable by phase-contrast microscopy at 7 to 8 h and was severe by 24 h (Rubenstein & Price, 1983).

The uptake of norepinephrine by PC12 cells has been characterized by Greene & Rein (1977a, b). Like neurons of the sympathetic nervous system, PC12 cells possess a high-affinity,
A saturable uptake mechanism which is energy- and Na+-dependent and is blocked by the inhibitor desmethylimipramine (DMI). We followed the methods of Greene & Rein (1977a) in assessing the effect of HSV-1 infection on norepinephrine uptake. In brief, at various intervals during the course of HSV-1 or mock infection, cultures were aspirated free of medium and washed once with 1 ml of freshly prepared KRH (HEPES-buffered modified Krebs-Ringer saline containing 125 mM-NaCl, 4-8 mM-KCl, 1-3 mM-CaCl₂, 25 mM-HEPES, 1-2 mM-MgSO₄, 1-2 mM-KH₂PO₄, 5-6 mM-glucose, and 1 mM-sodium ascorbate, adjusted to a final pH of 7.3 with NaOH and prewarmed to 37 °C); these and other chemicals for the present studies, unless otherwise indicated, were purchased from Sigma. The cultures were then pre-incubated for 15 min in 1 ml of prewarmed KRH containing 0.1 mM-pargyline (a monoamine oxidase inhibitor) before the addition of 1 ml KRH with pargyline and also containing 5 μM-[^3H]norepinephrine (1 μCi/ml, sp. act. 48 Ci/mmol; New England Nuclear). Following a 5 min incubation, uptake medium was removed and the cultures were rapidly washed three times with ice-cold KRH. The cells were then scraped from the dish with successive 0.5 ml aliquots of 1 M-HCl and 1 M-NaOH and placed into scintillation vials containing 10 ml Instagel (Packard) for counting. The results are expressed as pmol[^3H]norepinephrine taken up per culture per 5 min.

The results of determining [^3H]norepinephrine uptake during the 24-h course of PC 12 HSV-1 infection are shown in Fig. 1. In both HSV-1-infected and mock-infected cultures, a rise in uptake occurred over the first 3 h of the experiment, which was very likely related to the medium change accompanying viral inoculation. However, no difference was noted between the virus-infected and control cultures. To assess the specificity of the preserved catecholamine uptake observed in infected cells, we measured [^3H]norepinephrine uptake in the presence of 10^-6 M-DMI (a gift of USV Pharmaceuticals Corp., Tuckahoe, N.Y., U.S.A.). In both HSV-1- and mock-infected cultures, this inhibitor reduced norepinephrine uptake to negligible levels (data not shown), indicating that catecholamine uptake during infection was indeed specific and not a spurious observation related to coincident alteration in membrane permeability or transport.

We next assessed storage and K+-associated release of endogenous catecholamines during infection. These aspects of catecholamine metabolism in uninfected PC12 cells have been characterized by Greene & Rein (1977a) and Ritchie (1979). PC12 cells synthesize and store dopamine and norepinephrine and respond to elevated K+ concentration by releasing these catecholamines into the medium, presumably as a result of depolarization and exocytosis. In the same experiment we assessed endogenous catecholamines in unstimulated and K+-stimulated PC 12 cells during the course of HSV-1 and mock infection. Our methods again followed those developed by Greene & Rein (1977a). In brief, at various time intervals after inoculation, cultures were washed twice with prewarmed KRH, and then exposed to either KRH alone or 'releasing medium' (KRH supplemented with 51.5 mM-KCl). Following the addition of KRH alone, PC12 cells were immediately harvested with a rubber policeman, and pelleted in a Beckman microfuge. The pellet was suspended in 30 μl 0.2 M-perchloric acid containing 10 mM-dithiothreitol and frozen at -70 °C until used for catecholamine measurement. Upon exposure to 'releasing medium', cells were incubated for 15 min before harvesting in an identical manner. Samples were frozen and thawed three times, disrupted by ultrasonication for 5 s at setting 2 of a Branson Model 185 sonifier, and centrifuged at 8000 g for 3 min. An aliquot of the supernatant was used for catecholamine analysis by the method of Saller & Zigmond (1978). Samples were added to tubes with 10 μl 0.2 M-perchloric acid containing 40 mM-MgCl₂. Blanks contained 40 mM-CaCl₂ in place of MgCl₂. Twelve μl of reaction mixture [500 mM-Tris-acetate buffer pH 8.8, 5 mM-EGTA, 50 mM-dithiothreitol, 0.18% bovine serum albumin, 1.25 mM-pargyline hydrochloride, 20 units catechol-O-methyltransferase, 1 μl 0.025 M-sulphuric acid containing 1 μM-S-adenosyl-L-methionine (SAM), and 2 μl (1 μCi) [3H]SAM (sp. act. 48 Ci/mmol, New England Nuclear) in 0.025 M-sulphuric acid] was added to each sample. The samples were incubated at 30 °C for 30 min and the reaction was terminated by placing the tubes in an ice-bath and adding 10 μl 20% (w/v) phosphotungstic acid made up in 1.2 M-HCl. The tubes were then centrifuged at 8000 g and 36 μl of the supernatant was spotted on LK6DF thin-layer chromatography (TLC) plates (Whatman). The plates were dried and developed in the dark in a chloroform : methanol : ethylamine solution (16 : 3 : 2). After the plates were developed and dried,
Fig. 1. Norepinephrine uptake in HSV-1-infected PC12 cells. [3H]Norepinephrine uptake was determined in HSV-1-infected (●) and mock-infected (○) PC12 cells at the designated intervals after inoculation. Each point represents the mean of determinations on two separate cultures. Because of the closeness of the data points, error bars were not included, but in each case the standard deviation was within 10% of the mean value with the exception of the baseline and 1-h points in mock-infected cultures. No significant difference (P > 0.05) was noted at any point between infected and uninfected cultures as determined by Student’s t-test.

Fig. 2. Dopamine content of unstimulated and K+ -stimulated PC12 cells during the course of HSV-1 infection. Endogenous dopamine content of HSV-1-inoculated (●) and mock-infected (○) PC12 cells was assessed during the course of infection while they were maintained in standard medium. In parallel, cell dopamine was assessed in HSV-1-infected (■) and mock-infected (□) cultures 15 min after exposure to depolarizing medium containing 51.5 mM-KCl. Each point represents the mean of triplicate determinations and in each instance no significant difference was found between uninfected and infected cultures. Standard deviations were less than 6% of mean results.

The results of assessing dopamine content of PC12 cells over the 24-h course of HSV-1 infection are shown in Fig. 2. No difference in endogenous dopamine storage was detected between HSV-1- and mock-infected cultures. Similarly, as also shown in the figure, in the presence of high K+ concn. in the medium, dopamine was depleted from both HSV-1- and mock-infected cells in parallel; in both cases about 25% of endogenous stores were lost at each interval tested. Findings in the case of norepinephrine, which is present in lower concentrations than dopamine in PC12 cells, were similar with no detectable difference in either baseline or K+ -stimulated cellular stores between HSV-1- and mock-infected cells over the 24 h of observation (not shown).

We thus found remarkable preservation of specific catecholamine uptake, of endogenous catecholamine stores and of K+ -stimulated release during productive HSV-1 infection of the PC12 cell line. Despite conversion of these cells to virus production and the development of marked morphological cytopathology, there was sparing of these specific neuronal functions. Altered macromolecular synthesis and membrane composition (for review, see Spear & Roizman, 1980) seemingly left these systems intact, allowing specific transport of [3H]norepinephrine, preserving catecholamine stores and sparing depolarization-stimulated exocytosis of the neurotransmitters. We additionally tested the question of whether preservation of these cell
functions might have been due to differences between the cell body and neurites of PC12 cells in their alteration by infection. Thus, it might be hypothesized that the neurites, because of their progressive distancing from the cell body as they extend outwards, may function as a separate 'compartment' during infection. Indeed, we have observed morphological delay in neurite cytopathology in HSV-infected neurons (Price et al., 1982) and PC12 cells (Rubenstein & Price, 1983). To test this question, we infected NGF-treated PC12 cells maintained in suspension in flasks without collagen substrate, under which condition neurites do not develop. Data from these studies are not shown, but, in brief, we found that baseline [3H]norepinephrine uptake was reduced compared to neurite-bearing cells but that again no difference was found between infected and uninfected PC12 cells over the 24 h of the experiment. Similarly, endogenous dopamine and norepinephrine stores were preserved equally in infected and uninfected cells. Thus, infection failed to perturb these functions in the cell body, and therefore the findings in NGF-treated PC12 cells maintained on collagen were not simply due to sparing or delay in HSV-1-induced alteration of neurites.

These results underscore the selectivity of altered cellular metabolism within HSV-1-infected cells. Previous studies of other aspects of neurotransmitter metabolism within infected PC12 cells have shown how variably and unpredictably cellular functions may be altered during infection. In the instance of both acetylcholinesterase and choline acetyltransferase, which catalyse the degradation and synthesis of the neurotransmitter acetylcholine, we have noted a relatively early decline in enzyme activities (Rubenstein & Price, 1983). On the other hand, activity of tyrosine hydroxylase, which provides the putative rate-limiting step in catecholamine synthesis, was observed to undergo a pattern of depression, reconstitution and subsequent depression, with each of these stages probably related to expression of viral gene products occurring late in the course of infection (R. Rubenstein & R. W. Price, unpublished results). The significance in vivo of these observations in the pathogenesis of clinical symptoms and signs of acute herpetic infection remains uncertain. In the case of catecholamine uptake and release, the present observations suggest that infected neurons may retain a significant measure of functional capacity for a prolonged period during the course of infection.

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