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Effect of n-Butyrate on the *In Vitro* Reactivation of Latent Herpes Simplex Virus

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SUMMARY

n-Butyrate (n-BTA) increased the rate and number of infectious units produced in the *in vitro* reactivation of latent herpes simplex virus. While the mechanism of action of n-BTA is obscure, a continuous presence of n-BTA is necessary for its inductive effect.

Key Words: n-butyrate, in vitro reactivation, latent Herpes Simplex Virus

INTRODUCTION

n-Butyrate (n-BTA), a 4-carbon fatty acid, was found to be a potent inducer of the early antigen, of viral capsid antigen and to increase viral DNA synthesis in the Epstein-Barr Virus (EBV) producing lymphoma cell lines, P3HR-1 and B95-8. In the non-producer Raji line, n-BTA induced the early antigen without increasing viral DNA synthesis (Baringer & Swoveland, 1973). It is likely that the inhibitory function of n-BTA on cellular DNA synthesis is responsible for the activation of latent EBV in producer lines (Saemundsen, *et al.*, 1980).

Similar to EBV, herpes simplex virus (HSV) establishes a latent infection in both the sensory or autonomic ganglia, and central nervous system in humans (Baringer and Swoveland, 1973; Bastian, *et al.*, 1972; Fraser, *et al.*, 1981). Although the whole HSV genome is known to be present in a latent form, the nature of latent HSV is not yet known. It is, however, well known that reactivation of latent HSV in neurons leads to recurrent HSV diseases in the surface tissues of the body (Stevens, 1975). Therefore, many studies have been done in attempts to understand the mechanisms of the reactivation of latent virus, yet the molecular events are unknown (Stevens and Cook, 1974; Green, *et al.*, 1981; Galloway, *et al.*, 1982; Youssoufian, *et al.*, 1982). In the present study, we have investigated the effect of n-BTA on the *in vitro* reactivation of latent HSV in latently infected trigeminal ganglia of mice. This study was done in view of n-BTA's ability to induce the reactivation of latent EBV (Luca, *et al.*, 1979) and to induce differentiation in the Friend erythroleukemia system (Leder, et al., 1975).

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MATERIALS AND METHODS

Virus and Cells

HSV-1, F strain, obtained from the American Type Culture Collection (Rockville, MD), was propagated in Vero cells, and the viral titer was adjusted to 1.0×10^7 plaque forming units (PFU) per milliliter. The stock virus was stored at $-75°C$. Vero cells (ATCC, Rockville, MD) were grown in Eagle's minimal essential medium (E-MEM) supplemented with 5% fetal bovine serum (FBS).

Chemical compound

n-BTA was obtained from the Sigma Chemical Co. (St. Louis, MO). Proper concentrations of n-BTA were made in E-MEM supplemented with 5% FBS, and the solutions were filtered for sterilization before use.

Animals

Ninety-five, inbred albino male mice (BALB/c strain, 7 weeks old; Bantin and Kingman, Fremont, CA) were maintained for one week in the vivarium at the UCLA Medical Center. Five were housed in each cage.

Establishment of latent HSV infection in mice ganglia

Under pentobarbital anesthesia (60mg/kg, intraperitoneal injection), the corneas of both eyes of each mouse were scarified in a cross-hatched pattern with a 30-gauge needle. Ten microliters (μl) of HSV-1 was applied into the lower cul-de-sac of each eye with a gentle massage. Our preliminary study indicates that this ocular infection results in the development of latent HSV-1 infection in 100% of the trigeminal ganglia of inoculated animals.

Determination of the effect of n-BTA on the *in vitro* reactivation of latent HSV

Four weeks after the viral inoculation the animals were sacrificed and the trigeminal ganglia removed and placed in explant culture in the presence of 0.1, 0.25, 0.5, 1.0, or 2.0 mM of n-BTA for one, two, or three days. The ganglia were then washed twice with Dulbecco's phosphate buffered saline (PBS), homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY), and sonicated using a Sonifier (Branson Sonic Power Co., Plainview, NY). The titer of the reactivated virus in the homogenates was determined by a plaque assay in Vero cell monolayers. Each group included 7 to 9 ganglia. In an effort to study whether a continuous presence of n-BTA is necessary for its effect on the latent HSV, an additional 40 ganglia were placed in culture in the presence of 0.1, 0.25, 0.5, 1.0, or 2.0 mM of n-BTA for 6 hours. The n-BTA containing medium was then exchanged with fresh medium followed by 18 hours additional culture. The ganglia were then processed for the determination of the reactivated latent HSV.

Yield reduction assay

Confluent Vero cell monolayers were infected with HSV-1 at a multiplicity of infection (m.o.i.) of three for 1 hour, with intermittent rocking at 15 min intervals, and then washed twice with PBS. Medium, with or without n-BTA (0.1, 0.25, 0.5, 1.0, or 2.0 mM), was then added in triplicate to the appropriate Petri dishes. All cultures were incubated at 37°C for 12, 24, or 36 hours in a CO_2 incubator. They were then gently washed twice with PBS thereby preventing carry-over of n-BTA to the plaque assay. New n-BTA-free medium was then added. The cells were frozen and thawed three times, collected, and centrifuged. Viral titers in the supernatant were assayed in Vero cell monolayers by an ordinary plaque assay technique (Rapp, 1963).

RESULTS

Effect of n-BTA on the reactivation of latent HSV-1 *in vitro*

To observe the *in vitro* reactivation of latent HSV

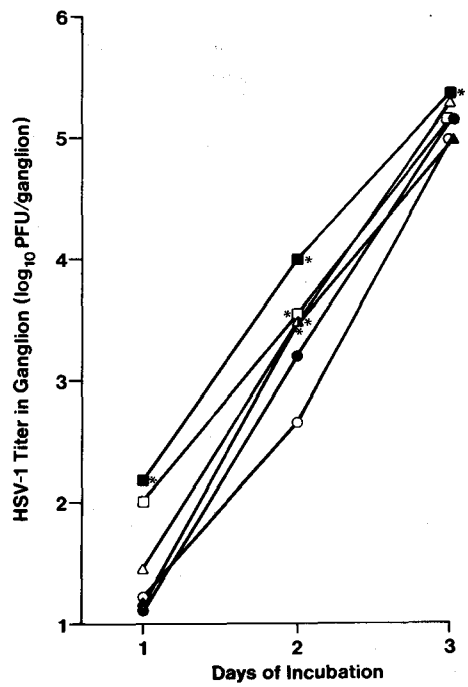

Fig. 1. Effect of n-BTA on the *in vitro* reactivation of ganglionic latent HSV-1. After the establishment of latent HSV-1 infection in mice as described in the text, the mice were sacrificed and the trigeminal ganglia were removed. The ganglia were then cultured in the medium containing 0.0 (○—○ Control group), 0.1 (●—●), 0.25 (△—△), 0.5 (▲—▲), 1.0 (□—□), 2.0 (■—■) mM of n-BTA for 1, 2, or 3 days. At the end of culture, the ganglia were washed, homogenized, and centrifuged. The viral titers were determined from the supernatants of the homogenates by using an ordinary plaque assay technique. Each value represents the average of 7 to 9 ganglia assayed individually. *Significantly different ($p<0.05$) from the control group (student t-test).

and daily change of virus titer in the ganglia, the trigeminal ganglia were cultured in drug-free medium for zero to three days. When the ganglia were homogenized immediately after removal (zero day culture) without allowing time for viral reactivation, the homogenates did not show any infectious virus (data not shown). One day culture in culture medium stimulated reactivation and infectious virus was isolated from 38% of tested ganglia. Two or three days culture in drug-free medium, however, allowed the isolation of infectious virus from all ganglionic homogenates (Fig. 1).

To determine the effect of n-BTA on influencing the *in vitro* reactivation of latent HSV, the gaglia were cultured immediately after removal from the mice in medium containing 0.1, 0.25, 0.5, 1.0, or 2.0 mM n-BTA for 1, 2, or 3 days. On day 1 postculture, the number of ganglia containing reactivated HSV was increased by n-BTA; 50-87% of ganglia with infectious HSV, and the titer of reactivated HSV was significantly raised only by 2.0mMn-BTA. When the ganglia were cultured for 2 days in the presence of various concentrations of n-BTA, the amount of infectious HSV in ganglia was significantly increased by n-BTA by a dose-dependent fashion and all ganglia showed infectious HSV. On day 3 postculture, the titers of infectious HSV in ganglia were not altered by the presence of 0.1-1.0mM n-BTA. The presence of 2.0mM n-BTA in culture medium, however, significantly increased the viral titer in ganglia (Fig. 1).

To study whether a continuous presence of n-BTA is necessary for its inductive effect, we have cultured ganglia in media containing 0.1-2.0mM n-BTA for 6 hours and changed the media with drug-free medium followed by 18 hours additional culture. Then the ganglia were washed and homogenized to determine the viral titer. The number of ganglia with infectious HSV and titers of virus in these groups were compared with those of groups continuously cultured for 24 hours in the presence of n-BTA. As shown in Figure 2, early removal of n-BTA from the culture medium did not allow it to show an inductive effect. The number of ganglia containing infec-

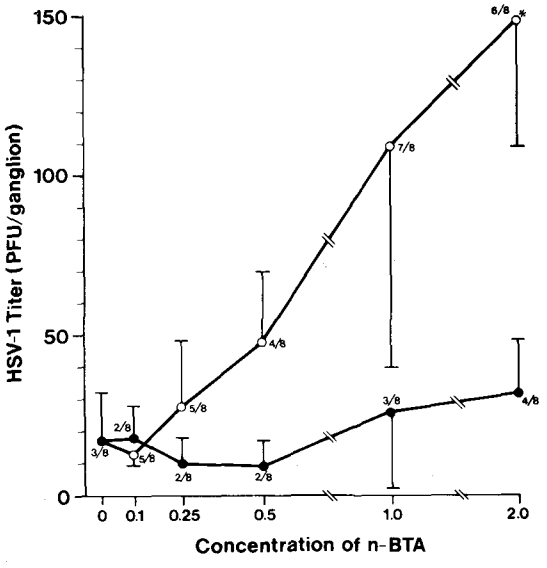

Fig. 2. Time-dependent effect of n-BTA on the reactivation of ganglionic latent HSV-1. The 40 latently infected ganglia were cultured in the presence of various concentrations of n-BTA for 6 hours. The n-BTA containing medium was then exchanged with fresh medium followed by 18 hours additional culture (●—●). Other 40 ganglia were cultured in n-BTA solution for 24 hours (○—○). The ganglia were then processed for the determination of the reactivated HSV-1 as described in Figure 1. Nominator: number of ganglia with reactivated HSV. Denominator: number of ganglia tested.

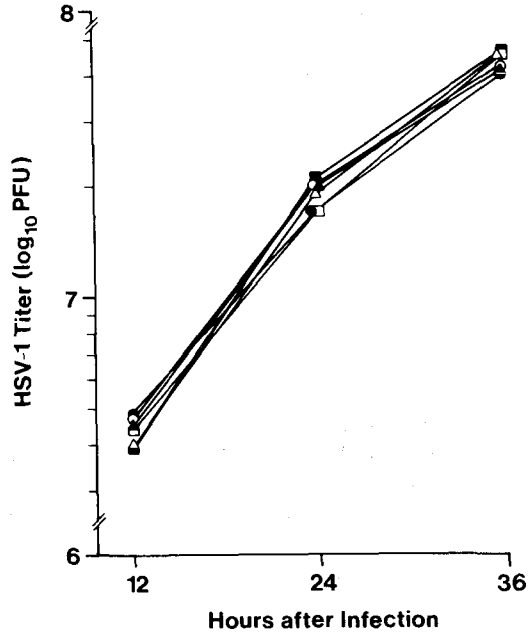

Fig. 3. Effect of n-BTA on the replication of HSV-1. Vero cell monolayers were infected with HSV-1 at m.o.i. of 3 for 1 hour. The cultures were washed and medium containing 0.0 (○—○, control), 0.1 (●—●), 0.25 (△—△), 0.5 (▲—▲), 1.0 (□-□), 2.0 (■—■) mM of n-BTA was added in triplicate. All cultures were incubated for 12, 24 or 36 hours, and then gently washed twice with PBS thereby preventing carry-over of n-BTA. New fresh medium was added and viral titers were assayed.

tious virus and the titers of reactivated HSV were not altered by the early removal of n-BTA, while the continuous presence of n-BTA notably increased both of them.

Effect of n-BTA on the replication of HSV-1

As shown in Figure 3, the replication of HSV-1 in the monolayers of Vero cells was not influenced by various concentrations of n-BTA which affected the reactivation of ganglionic latent HSV-1.

DISCUSSION

Our results indicate that n-BTA may accelerate the reactivation process of latent HSV in the trigeminal ganglia. In the presence of n-BTA, the reactivated infectious virus appeared earlier than in controls and the amount of reactivated virus was significantly increased in ganglia. Yet, one can argue that n-BTA may simply induce additional cycles of viral replication rather than an increase in the reactivation process, or it might reduce the time necessary for HSV to undergo one cycle of replication. In order to study these possible effects of n-BTA, we initiated a time-course experiment (Figure 3). When the monolayers of Vero cells were inoculated with HSV-1 in the presence of n-BTA, the viral yield was not altered at 12, 24, and 36 hours postinfection. These results negate the proposed questions possibly attributable to the effects of n-BTA.

While the mechanism of n-BTA action or effect remains obscure, it seems that a continuous presence of n-BTA in culture medium might be a prerequisite for its inductive effect. n-BTA has been shown to induce morphological changes and growth inhibitions in cultured cells (Prasad and Hise, 1971). n-BTA also elevates the intracellular cAMP levels in mouse neuroblastoma cells (Prased, *et al.*, 1973). Since increased intracellular cAMP levels might be linked to the enhanced reactivation process of latent HSV (Park *et al.*, unpublished data), n-BTA may exert its inductive effect by elevating intracellular cAMP in latently infected neurons. Since viral DNA sequences can be associated with histones in cell chromosomes, and n-BTA induces the hyperacetylation of the histones, this hyperacetylation may play a role in the initiation and the resultant increased reactivation process of the latent virus (Chestier and yaniv, 1979). The exact mechanism of the function of n-BTA on latent HSV is not known and should be explored in the near future.

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=국문초록=

잠재성 Herpes Simplex Virus의 재활성화에 대한 n-Butyrate의 효과

고려대학교 의과대학 약리학교실 · 로스엔젤레스 캘리포니아대학교 치과대학 구강생물학교실
천연숙, 박노희

4-carbon fatty acid인 n-Butyrate(n-BTA)는 herpes virus의 일종인 Epstein-Bar virus(EBV)에 작용해서 잠복형인 EBV를 활성형태로 유도시키는 것으로 알려져 있다. 고로 본 실험에서는 mouse의 삼차신경절에 잠복하고 있는 HSV의 재활성화에 대한 n-BTA의 효과를 실험 관찰하였다. Pentobarbital로 마취시킨 mouse의 양쪽눈 각막을 30 gauge 주사바늘로 scarify한 후에 type I HSV(HSV-1) 10μl(1×10^5 plaque-forming units)를 각각 점안 감염시켰다. virus를 감염시킨 4주 후에 mouse의 삼차신경절을 적출하여 시험관 내에서 조직배양을 시행하였다. 조직배양시에 0.1, 0.25, 0.5, 1.0 그리고 2.0mM농도의 n-BTA를 첨가하였으며 1일, 2일, 3일간 각각 배양한 후 신경절을 연마하여 연마액내의 HSV-1 titer를 Vero cell monolayer에서 plaque assay로 측정하였다.

1) n-BTA첨가군은 잠재성 HSV가 대조군에 비하여 현저하게 빨리 재활성화 되었고 재활성화되는 virus의 양도 현저히 증가되었다.
2) 24시간을 계속해서 n-BTA 각 농도를 첨가해서 배양한 군은 n-BTA 6시간 첨가 배양하고 새로운 배양액으로 갈아서 18시간 배양한 군에 비해 잠재성 virus의 재활성화가 현저히 증가되었다.
3) Ganglionic latent HSV-1의 재활성화에 영향을 미치는 각 농도의 n-BTA는 Vero cell의 monolayer에서의 HSV-1의 번식에는 아무런 영향을 미치지 않았다.