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Short Communication

Chronic cocaine exposure in the SCID mouse model of HIV encephalitis

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ABSTRACT

Clinical and preclinical evidence suggests that cocaine exposure hastens progression of the HIV disease process. An established active, euphoric dose of cocaine (20 mg/kg) was administered to SCID mice according to a regimen consistent with exposure to the drug by cocaine-abusing HIV-infected patients to determine the effects of cocaine on four previously established pathological characteristics of HIV encephalitis: cognitive deficits, fatigue, astrogliosis, and microgliosis. Mice were intracranially inoculated with either HIV-infected, or uninfected macrophages and then injected with either cocaine or saline in a 2 (Infection)×2 (Cocaine) factorial design. Cognition was assessed by acquisition and retention of a spatially cued learning task. Fatigue was assessed by monitoring motor activity following a 2 min forced swim. Mice were then sacrificed to determine the extent of astrogliosis and microgliosis in the four groups. Results indicated that in comparison to uninfected controls, HIV positive mice had increased astrogliosis and microgliosis, cognitive deficits, and recovered more slowly from fatigue. However, despite evidence that the cocaine exposure regimen activated the central nervous system and had long-term CNS effects, the drug did not alter the behavioral or the neuropathological deficits noted in HIV-infected SCID mice.

Current estimates indicate that a high proportion of HIV-infected individuals abuse drugs (HIV/AIDS, 2004) such as cocaine. In addition to being an HIV infection risk factor (Mccoy et al., 2004), cocaine appears to detrimentally modify the HIV disease process (Goodkin et al., 1998). Further, cocaine directly influences the immune system as demonstrated by increases in TNF alpha and IL-6 production after cocaine exposure and excessive exposure to these pro-inflammatory cytokines could be detrimental to brain tissue (see Review; Tyor and Middaugh, 1999). Thus, in addition to reducing HIV medication compliance (Arnsten et al., 2002), these findings suggest that cocaine abuse is capable of accelerating HIV-associated complications.

HIV-associated dementia (HAD) occurs commonly in HIV infected patients (McArthur, 1990; McArthur et al., 1993) and is an AIDS defining illness. SCID mice, inoculated with HIV-infected macrophages, model many features of HAD such as gliosis and HIV-infected mononuclear phagocytes (Avgeropoulos et al., 1998b; Persidsky et al., 1996; Tyor et al., 1993) as well as cognitive impairments (Avgeropoulos et al., 1998b; Griffin et al., 2004). As such, the SCID mouse model of HIV encephalitis can help elucidate the interactive effects of

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cocaine and HIV on indexes of neuropathological damage and behavioral deficits (Tyor and Middaugh, 1999).

The primary objective of the present study was to examine the influence of chronic cocaine exposure in HIV-positive SCID mice on the behavioral measures, cognition and motor fatigue, and on the neuropathological features, astrogliosis and microgliosis. Because cocaine abuse was reported to exacerbate self-reported cognitive impairment in HIV seropositive patients (Avants et al., 1997), our hypothesis was that chronic cocaine exposure would exacerbate the cognitive impairment previously reported in HIV-infected SCID mice (Avgeropoulos et al., 1998b; Griffin et al., 2004). In addition, we hypothesized that histopathological features of the HIV encephalitis (Avgeropoulos et al., 1998b; Persidsky et al., 1996; Tyor et al., 1993) would also be worsened by chronic cocaine exposure.

Subjects. Male CB-17 SCID mice (4 weeks old) were obtained from Charles River Laboratory (Wilmington, MA). They were singly housed in micro-isolator cages with free access to food and water for 1 week prior the experiment as previously described (Avgeropoulos et al., 1998b; Griffin et al., 2004). All procedures were conducted in AAALAC-approved facilities, were approved by the Institutional Animal Care and Use Committee, and were consistent with the guidelines of the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996).

Mouse inoculation with HIV-infected and uninfected macrophages. Although there is evidence mice can be non-productively infected with HIV (Avgeropoulos et al., 1998a) to better approximate productive infection during HIV encephalitis, mice were inoculated with HIV-infected human macrophages. This xenograft endures 4–6 weeks (Tyor et al., 1993) and provides the opportunity to evaluate behavioral manifestations of HIV encephalitis. To control for the effects of human macrophages themselves, a separate group of mice were inoculated with uninfected macrophages. The cell culture and inoculation procedures for the current study were identical to recent reports (Avgeropoulos et al., 1998b; Cook et al., 2005; Griffin et al., 2004). Briefly, 10^8 purified primary human macrophages were cultured in the presence of monocyte colony stimulating factor (M-CSF). Half of the cells were infected with HIV-1_{ADA} virus at a multiplicity of infection of 0.2 viral particles per cell while the other half remained uninfected. After 2 weeks of culture, mice were inoculated with either 1×10^5 infected cells or with uninfected cells resuspended in 30 μL phosphate buffered saline. Cells were delivered over 25 s using a freehand injection into the right frontal lobe, behind the orbital sinus, using a syringe fitted with a collar to control depth to 4 mm below the skull surface. The xenograft placement corresponds to plate 160 in the brain atlas by Sidman et al. (1971). The procedure yields reproducible xenografts within and across experiments.

Experimental procedure. The experiment was conducted over a 9-week period and consisted of 4 groups of mice distinguished by HIV status (i.e., whether the inoculated macrophages were HIV infected or not) and drug treatment (IP injections of Cocaine, 20 mg/kg or equivalent volumes of Saline Vehicle): HIV negative+Vehicle (NV), HIV negative+Cocaine (NC), HIV positive+Vehicle (PV) and HIV positive+Cocaine (PC). Vehicle (0.9% saline) or cocaine (20 mg/kg) was injected intraperitoneally at a volume of 0.01 ml/g bodyweight daily (~1000 hr) Monday through Friday during Weeks 1 through 5. Several mice were excluded from the final analysis based on inconclusive histopathological evidence of HIV infection ($n=8$). The final group sizes for the 2 (HIV)×2 (Cocaine) factorial design were: NV ($n=12$), NC ($n=11$), PV ($n=9$), PC ($n=8$).

The cocaine exposure regimen was designed to provide drug exposure consistent with that of a cocaine abusing, HIV positive patient, with exposure beginning prior to HIV infection and continuing after HIV infection. Drug holidays were incorporated into the model to reflect the intermittent use suggested by studies on treatment seeking individuals (e.g., Grabowski et al., 2000; Myrick et al., 2001a,b). Further, since humans abuse cocaine for its euphoric value (Dackis and O'Brien, 2001), an essential part of the model was to use a dose of cocaine that activates the central nervous system and is rewarding for mice.

Cocaine effects on motor activity of SCID mice. To select a cocaine dose for the primary study, locomotor activity was assessed over a 60-min time period following IP injections of 0, 10, 20, 30 and 40 mg/kg doses of cocaine in SCID mice ($n=6$ per dose) using a previously described procedure (Griffin et al., 2004). Cocaine induced seizures at the two highest doses and death for most of the mice injected with the 40 mg/kg dose. The two lower doses did not induce seizures but increased activity above vehicle levels; 55% for the 10 mg/kg dose and 61% for the 20 mg/kg dose. The 20 mg/kg dose was selected for further study because: 1) in addition to its CNS activation for the SCID mice, it stimulates activity in several mouse strains (Griffin and Middaugh, 2006; Kuzmin et al., 2000; Tolliver and Carney, 1994), 2) there is solid evidence of its hedonic value in mice using place conditioning studies (Szumlinski et al., 2002, 2004) and self-administration experiments (Griffin and Middaugh, 2003), and 3) the larger peak concentrations following i.p. injection of 20 mg/kg would prolong the xenograft's exposure to cocaine, an important consideration given cocaine's half life of 11 to 22 min in mice (Azar et al., 1998; Benuck et al., 1987; Reith et al., 1987).

Cocaine exposure did not alter locomotor activity in HIV-positive mice challenged with cocaine. Motor activity of the four groups of mice defined by inoculation with either the HIV-infected, or uninfected macrophages, and whether injections were saline or cocaine (20 mg/kg) was assessed at weekly intervals, two prior to (i.e., Weeks 1 and 2), and two after inoculation (Weeks 3 and 5). Activity assessed on Week-1 indicated that the first exposure to cocaine increased activity approximately 74% over that of vehicle treated controls. The second activity test on Week 2 (i.e., after 7 daily vehicle or cocaine injections) indicated approximately 45% elevated activity for cocaine compared to vehicle-injected mice. A 2 (Cocaine Dose)×2 (Week) ANOVA provided statistical confirmation that cocaine stimulated motor activity of SCID mice [$F(1,36)=16.8$, $p<0.001$] prior to their inoculation with HIV infected or uninfected macrophages.

After inoculation with either HIV-infected or uninfected macrophages, cocaine continued to stimulate motor activity with the degree of stimulation greater for the third test 1 week after inoculation (40% increase) than the fourth test 2 weeks after inoculation (16% increase). A significant