THE EFFECTS OF COCAINE AND STRESS ON LYMPHOCYTE PROLIFERATION IN RATS

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Abstract

Title of Thesis: The effects of cocaine and stress on lymphocyte proliferation in rats

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The effects of cocaine, stress, and the combination of cocaine and stress on cellular immune proliferation stimulated by mitogens Con A and LPS were examined in rats. Hypothalamic dopamine and norepinephrine were measured to examine whether catecholamine levels relate to changes in immune function in this paradigm. Cocaine, stress, and cocaine plus stress decreased cellular immune function to Con A mitogen compared to a control group. Hypothalamic dopamine levels were inversely related to immune proliferation to LPS mitogen. The results are discussed in terms of the deleterious effects of cocaine and stress, and possible mechanisms for cocaine and stress-induced immune suppression.

The Effects of Cocaine and Stress

on

Lymphocyte Proliferation in Rats

by

Lorenzo Cohen

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INTRODUCTION

Cocaine is a widely used drug that, because of its addictive properties and possible health consequences, has received a great deal of research attention (Bagarasa & Forman, 1989; Centers for Disease Control, 1991a; Centers for Disease Control, 1991b; Johanson, 1988; 1986). Among the health-relevant effects of cocaine is its potential to suppress immune function, but reports on this topic are inconsistent (Bagarasa & Forman, 1989; Di Francesco et al., 1990; Havas, Dellaria, Schiffman, Geller, & Adler, 1987; Klein, Newton, & Freidman, 1988; Van Dyke, Stesin, Jones, Chuntharapai, & Seaman, 1986; Watson, Murphy, Elsohly, Elsohly, & Turner, 1983). Stress, which is associated with substance abuse, reliably suppresses immune function (Ben-Eliyahu, Shavit, & Yirmiya, 1990, 1991; Batuman, Sajewski, Ottenweller, Pitman, & Natelson, 1990; Cunnick, Lysle, Armfield, & Rabin, 1988; Keicolt-Glaser & Glaser, 1991; Lysle, Lyte, Fowler, & Rabin, 1987; Shavit & Martin, 1987). If both cocaine and stress suppress immune function, then their combination could have an even greater effect (Raygada & Baum, 1991; Mokler, Correla, & Novotny, 1991). The present experiment was designed to examine effects of cocaine administration and stress on cellular immune function in rats.

Cocaine and Immune Function. The effects of cocaine on cellular and humoral immunity have been studied but results are not clear. Some studies have reported that cocaine increases some measures of immune function or has no effect. Havas et al. (1987) reported that intraperitoneal administration of cocaine (50 mg/kg, 3 times) increased plaque forming cells (PFC) in male mice, but susceptibility to infection by *S. pneumoniae* was not altered. In addition, Havas et al. (1987) reported that cocaine had no effect on tumor growth or the rate of survival of mice injected with tumor cells.

Van Dyke et al. (1986) reported that intravenous administration of cocaine to humans (0.6 mg/kg) caused a rapid increase in natural killer (NK) cell number and lytic ability. Changes in NK cell activity were temporally related to circulating cocaine levels, and NK cell numbers and activity returned to baseline after 80 min (Van Dyke, et al., 1986). Careful interpretation of these results is needed due to the fact that they included only eight subjects between 21-31 yr. Further the study was broken up into 15 separate sessions and the results combined in the data analysis.

Similar studies report that cocaine increases some indices of immune function while decreasing others. Bagarasa and Forman (1989) found that intraperitoneal cocaine (1.25, 2.5 mg/kg-10 days) at low doses caused an

increase in PFC response in male Fisher rats, but at higher doses (5 mg/kg-10 days) PFC response was suppressed. Analysis of splenic subpopulations revealed a decrease in total T cells, a reciprocal increase in the B cell population, and macrophage and T cell subsets were unchanged (Bagarasa & Forman, 1989). Faith and Valintine (1983) reported that intraperitoneal administration of cocaine (30-60 mg/kg) to mice for up to 14 days increased delayed type hypersensitivity (DTH) and increased splenocyte proliferation to T cell mitogens, but decreased proliferation to B cell mitogen.

Other studies have reported consistent decreases in immune function with cocaine administration. Klein, Newton, and Friedman (1988) found that cocaine combined with Con A *in vitro* suppressed lymphocyte proliferation in a dose-dependent fashion for mice splenocytes and human lymphocytes. Watson et al. (1983) also reported that after oral administration of cocaine (15, 30, 60, 100 mg/kg-4 days) to mice, DTH and PFC responses decreased. Di Francesco et al. (1990) found that acute and chronic cocaine administration (intraperitoneal 1 mg/kg-1 day, or subcutaneous 1 mg/kg-7 days) decreased NK cell function and T cell response, with recovery to baseline taking longer after chronic drug administration. Further, both acute and chronic cocaine administration decreased resistance to influenza virus (Di Francesco et al., 1990).

These findings appear to be inconsistent but do not necessarily represent conflicting findings. The different experimental procedures, methods of cocaine administration (oral, intraperitoneal, subcutaneous, intravenous), dosages used, durations of exposure, tests of immune function (*in vivo*, *in vitro*), as well as different gender and species, may be responsible for this confusing literature. Few of the studies cited used comparable immune measures to evaluate level of functioning. More importantly, these studies do not evaluate the effects of cocaine in the context of ongoing events that might be associated with cocaine use, such as stress.

Stress and Immune Function. Stress affects immune function in humans and animals, with stressors decreasing most indices of immune function (cf. Keller, Schleifer, & Demetrikopoulos, 1991; Keicolt-Glaser & Glaser, 1991). Monjan and Collector (1976) found that an acute stressor decreased lymphocyte-mediated cytotoxic response and proliferation in mice. In addition, other studies reported stress-related decreases in NK cell cytotoxicity and T cell proliferation in rats and mice (Batuman et al., 1990; Ben-Eliyahu et al., 1990; 1991; Cunnick et al., 1988; Lysle et al., 1987; Shavit et al., 1987). In 1990, Batuman et al. found that both acute stress (one 3 hour exposure to restraint plus intermittent shock) and chronic stress (daily

3 hour restraint plus intermittent shock exposure for 7 or 14 days) decreased the number of mononuclear cells, particularly CD8 cells, in mice. Further, they found decreased T-cell response to Con A and PHA. There was also a decreased production of IL-2 from T-cells stimulated with PHA.

Because drug use and stress may frequently co-occur (Wills, 1986), it is important to examine the independent as well as the combined effects of cocaine and stress on immune function. Recently Mokler, Correla, and Novotny (1991) studied the effect of cocaine and stressor exposure on humoral immunity in mice, crossing stress (daily one-hour noise exposure for 14 days) and intraperitoneal cocaine administration (5 mg/kg-14 days). Stress alone and in combination with cocaine administration decreased production of several immunoglobulins. There is no report indicating if cocaine alone had any effect on immunoglobulin levels (Mokler et al., 1991).

The present experiment was designed to extend Mokler et al.'s (1991) findings to examine cellular immune responses to continuous cocaine and acute stress administration in rats. The study used a cocaine by stress factorial design to examine *in vitro* mitogen stimulated splenocyte function. It was hypothesized that stress and cocaine would decrease lymphocyte proliferation compared to controls, and that stress and cocaine together would decrease proliferation

compared with controls and to the single treatment groups. Further, it was hypothesized that brain dopamine and norepinephrine levels would predict lymphocyte proliferation, because sympathetic arousal, specifically increased epinephrine, has been suggested as a primary mechanism of amphetamine and stress-induced immune suppression (Pezzone, Rush, Kusnecov, Wood, & Rabin, 1992).

METHODS

Animals. Subjects were 40 male Sprague-Dawley rats (Charles River Laboratories) weighing 250-300g at the beginning of the study. Animals were housed individually, kept on a 12 hr light dark schedule, and had continuous access to bland food (Purina Rat Chow) and water. Animals were quasi-randomly assigned (matching for initial body weight) to one of four groups: the Cocaine group received 70 mg/kg/24 hours cocaine HCl; the Cocaine+Stress group received 70 mg/kg/24 hours cocaine HCl plus two hours of restraint stress prior to sacrifice; the Stress group received saline plus two hours of restraint stress prior to sacrifice; and the Saline group received saline as a control. Cocaine and saline were administered by Alzet miniosmotic pumps (Model 2002, Alza Corporation, Palo Alto, CA.) for 6 days.

Procedures. Body weight was measured daily using Sartorius electronic balances (Model 1264MPBCD) programmed to provide

the mean of 10 separate weighings. Food consumption was measured every day by weighing food cups upon removal from each cage and again after cups were refilled. After a baseline period (5 days), animals were anesthetized by inhalation of metophane, an incision was made in the back between the shoulders, a miniosmotic pump (Model 2002, Alza Corporation, Palo Alto, CA.) was inserted, and the incision was closed with 9 mm clips. The minipumps delivered cocaine HCl or saline at a rate of approximately 0.5 ul/hr, providing continuous administration at a constant rate.

Stress manipulation. On the seventh day of cocaine or saline exposure, between 7 and 10 am, animals in the stress groups were immobilized in a separate room for 2 hours prior to sacrifice. The restraining device (Fisher Scientific, Centrap cage) held each rat in a normal standing position by adjustment of plastic "fingers" that fit around the animal's torso without squeezing, ensuring that the animals could not turn around. This technique has reliably produced stress-like increases in biochemical indices but with minimal apparent distress to the animals (Kant et al., 1983; Kant, Leu, Anderson, & Mougey, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992). Animals in the no stress conditions remained in the animal colony until sacrificed.

Sacrifice procedures. Animals were decapitated on the seventh day of either cocaine or saline exposure between 7 and 10 am. The spleen and brain were removed from each animal, and trunk blood was collected in heparinized tubes. The hypothalamus was dissected from each brain to measure DA and NE levels. Samples were frozen on dry ice and stored at -70^{0}C until later assay.

Immune measures. Spleens were removed under aseptic conditions and were placed in polypropoylene tubes containing 7 ml of Hepes enriched RPMI tissue culture medium (Gibco) that was supplemented with 2mM glutamine (Gibco), 50 ug gentamicin/ml (Gibco), and 10% fetal calf serum (Difco). This procedure was based on Cunnick et al. (1988). Briefly, a single cell suspension of splenocytes was prepared by gently pressing the spleens using the end of a 10 ml sterile syringe in a petri dish. The leukocytes were then counted using trypan blue exclusion criteria, and their concentrations were adjusted to 5 x 10^{6} cells/ml without further washing. Con A at 1.0 and 5 ug/ml and LPS at 5.0 and 10.0 ug/ml were prepared in supplemented RPMI and 100 ul were added in triplicate to the wells of a 96-well, flat-bottom, microtiter plate (Costar No. 3596), and 100 ul of supplemented RPMI were added to background culture wells. Then, 100 ul of adjusted spleen-cell suspensions were added to each well and the plates were incubated at 37°C in a

humidified incubator with 5% CO_2. Cultures were pulsed with 1 uCi tritiated thymidine (DuPont-New England Nuclear) in 50 ul of supplemented RPMI during the last 5 h of a 48-h incubation. The cultures were harvested using a PHD cell harvester (Model 200A, Cambridge Technologies Inc.) onto glass filter paper and the incorporation of tritiated thymidine was determined with a liquid scintillation counter (1218 RackBeta, LKB-Wallac) and was expressed as counts per minute (CPM). Analyses were conducted on the value of the mitogen-stimulated wells minus the background wells, where there was no mitogen stimulation.

Catecholamine assay. DA and NE were extracted from the hypothalamus by homogenizing the tissue in 0.01HCl, and the concentrations of the catecholamines were determined by a radioenzymatic procedure based on methods described by Weise and Kopin (1976) and Durrett and Ziegler (1980).

RESULTS

Cocaine and stress were associated with immune function in this study (see Table 1). A two-way analysis of variance revealed that cocaine exerted a marginally significant suppressive effect on lymphocyte proliferation to Con A at both mitogen levels, 1 ug/ml ($F(1,39) = 3.87$, $p < .057$), and 5 mg/ml ($F(1,39) = 3.65$, $p < .064$). Examination of the data (see Table 1) suggested excessively large variance for the

proliferative response to Con A mitogen, and closer inspection revealed that most of this variance was attributable to a few subjects. Therefore, the data were Windsorized (Wainer, 1982) by removing the two highest and two lowest values in each group (see table 1). A two-way analysis of variance of Windsorized data revealed significant effects for drug ($F(1,23) = 9.62$, $p < .006$), and stress ($F(1,23) = 8.5$, $p < .009$). The interaction term was not significant ($F(1,23) = 1.46$, $p < .24$). Newman-Keuls mean comparisons indicated that the three experimental groups (cocaine, stress, cocaine+stress) were significantly different from the control group ($p < .05$).

Insert Table 1 About Here

There were no significant effects for LPS at either level, suggesting that B cell function was not affected by these manipulations. No significant group differences were found for hypothalamic NE and DA (Table 1). However, a multiple regression analysis using group, hypothalamic NE, and hypothalamic DA as predictor variables revealed that hypothalamic DA levels were negatively related to B cell function, accounting for 19% of this variance ($F(3,36) = 4.99$, $p < .005$). A repeated measures analysis of variance revealed that food consumption on the first day decreased

significantly in the two cocaine treatment groups compared to the groups that did not receive cocaine ($F(1,39) = 3.49$, $p < .03$). This decrease in food consumtion was also present on the second day ($F(1,39) = 3.35$, $p < .03$), but disappeared on subsequent days. Body weight and food consumption were not significantly correlated with immune function.

DISCUSSION

These findings indicated that continuous cocaine administration, acute stress, and the combination of continuous cocaine administration plus acute stress had immunosuppressive effects, decreasing T cell proliferation in response to Con A mitogen in healthy male rats. Magnitudes of the effects for the treatment groups suggest that the immunosuppressive effects of cocaine and stress may be additive, but the interaction between stress and cocaine manipulations was not significant. Hypothalamic dopamine levels were inversely related to proliferative values so the catecholamine system may play a role in immune function. This interpretation is consistent with a study by Pezzone et al. (1992) who found that beta-receptor blockade prior to administration of amphetamine attenuated amphetamine-induced immunosuppression. Also, Strausbaugh and Irwin (1992) reported that central CRH administration reduced several immune parameters, and that B-receptor blockade attenuated

CRH-induced immune suppression as well (Irwin, Hauger, Jones, Provencio, & Britton, 1990).

Consistent with Mokler et al.'s 1991 report that stress independently and in combination with cocaine decreases immunoglobulin levels, stress and cocaine had immunosuppressive effects. These results extend Mokler et al.'s findings to include cell-mediated immunity. Further, cocaine was associated with decreases in lymphocyte proliferation, consistent with previous research (Di Francesco et al., 1990; Klein, et al., 1988; Watson, et al., 1983). Di Francesco et al. (1992) found that cocaine decreased *in vitro* release of IL-2, which could be a mechanism responsible for cocaine induced cellular immune suppression. Further, increases in sympathetic nervous system activity may be responsible for these changes, and future research should examine potential mechanisms (e.g., catecholaminergic) of this immune suppression and should evaluate behavioral and pharmacological variables that might attenuate this effect.

The results of this study have implications for the cocaine abusing segment of our population. Drug induced immune suppression may increase vulnerability to disease for people abusing cocaine. Further if cocaine abuse is accompanied by high levels of stress, an additive effect in decreasing one's immune system may result, leaving a person more vulnerable to health problems.

Table 1: Group means and standard errors for proliferation to Con A mitogen at 1 ug/ml, 5 ug/ml, and catecholamine data.
Total= all subjects in the sample; Windsorized= Windsorized data.

	Saline	Stress	Cocaine	Cocaine/Stess
Con A @ 1 ug/ml Total (n=10)	38,195± 8,710	29,021± 9,081	25,665± 7,524	12,435± 1,925
Con A @ 1 ug/ml Windsorized (n=6)	35,931± 6,368	19,729± 3,124	19,003± 2,942	12,229± 1,647
Con A @ 5 ug/ml Total (n=10)	111,658± 3,1469	90,031± 27,076	72,210± 21,541	38,419± 9,073
Con A @ 5 ug/ml Windsorized (n=6)	100,486± 31,250	64,882± 19,428	58,465± 16,725	32,656± 7420
Hypothalamic DA (n=10)	1.20± 0.21	1.16± 0.21	1.44± 0.22	1.62± 0.20
Hypothalamic NE (n=10)	3.30± 0.62	3.37± 0.59	4.26± 0.63	3.64± 0.62

REFERENCES

Bagarasa, O., & Forman, L. (1989). Functional analysis of lymphocytes subpopulations in experimental cocaine abuse. I. Dose-dependent activation of lymphocyte subsets. *Clinical Experimental Immunology*, **77**, 289-293.

Ben-Eliyahu, S., Shavit, Y., Yirmiya, R., & Liebeskind, J. C. (1991). Stress-induced suppression of natural killer cell cytotoxicity in the rat: A naltrexone-insensitive paradigm. *Behavioral Neuroscience*, **104**, 235-238.

Ben-Eliyahu, S., Shavit, Y., Yirmiya, R., & Liebeskind, J. C. (1990). Stress increases metastatic spread of mammary tumor in rats: Evidence for mediation by the immune system. *Brain, Behavior, and Immunity*, **5**, 193-205.

Batuman, O.A., Sajewski, D., Ottenweller, J.E., Pitman, D.L., & Natelson, B.H. (1990). Effects of repeated stress on T cell function in rats. *Brain, Behavior, and Immunity*, **4**, 105-117.

Centers for Disease Control (1991a). Current tobacco, alcohol, marijuana, cocaine use. *Journal of the American Medical Association*, **40**, 2061-2062.

Centers for Disease Control (1991b). Alcohol and other drug use among high school students - United States, 1990. *Journal of the American Medical Association*, **40**, 3266-3267.

Cunnick, J. E., Lysle, D. T., Armfield, A., & Rabin, B. S. (1988). Shock-induced modulation of lymphocyte responsiveness and natural killer activity: Differential mechanisms of induction. *Brain, Behavior, and Immunity*, **2**, 102-113.

Durrett, L.R., & Ziegler, M.G. (1980). A sensitive radioenzymatic assay for catecholamine drugs. *Journal of Neuroscience Research*, **5**, 587-598.

Di Francesco, P., Marini, S., Pica, F., Favalli, C., Tubaro, E., & Garaci, E. (1992). In vivo cocaine administration influences lymphokine production and humoral immune response. *Immunological Research*, **11**, 74-79.

Di Francesco, P., Pica,F., Croce, C., Favalli, C., Tubaro, E., & Garaci, E. (1990). Effect of acute or daily cocaine administration on cellular immune response and virus infection in mice. *Natural Immunity and Cell Growth Regululation*, **9**, 397-405.

Faith R.E., & Valintine J.L. (1983). Effects of cocaine exposure on immune function. *Toxicologist*, **3**(1), 56.

Havas, F. H., Dellaria, M., Schiffman, G., Geller, E. B., & Adler, M. W. (1987). Effect of cocaine on the immune response and host resistance in the BALB/c mice. *International Archives of Allergy and Applied Immunology*, **83**, 377-383.

Irwin, M., Hauger, R.L., Jones, L., Provencio, M., & Britton, K.T. (1990). Sympathetic nervous system mediates central corticotropin releasing factor induced suppression of natural killer cytotoxicity. *Journal of Pharmacology and Experimental Therapeutics*, **255**, 101-107.

Johanson, C.E. (1988). Behavioral studies of the reinforcing properties of cocaine. *NIDA Research Monograph Series*, **88**, 107-124.

Johanson, C.E. (1986). *Cocaine: New Epidemic.* In S.H. Snyder (ed.). *The Encyclopedia of Psychoactive Drugs*, **19**. New York, NY, Chelsea House Publishers.

Kant, G.L., Lenox, R.H., Bunnell, B.N., Mougey, E.H., Pennington, L.L., & Meyerhoff, J.L. (1983). Comparison of stress response in male and female rats: Petuitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. *Psychoneuroendocrinology*, **4**, 421-428.

Kant, G.L., Leu, J.R., Anderson, S.M., & Mougey, E.H. (1987). Effects of chronic stress on plasma corticosterone, ACTH and prolactin. *Physiological Behavior*, **40**, 775-779.

Keicolt-Glaser, J.K., & Glaser, R. (1991). Stress and immune function in humans. In R. Ader, D.L. Felten, & N. Cohen (eds.), *Psychoneuroimmunology*, pp. 849-864. Academic Press Inc., San Diego, California.

Keller, S.E., Schleifer, S.J., & Demetrikopoulos, M.K. (1991). Stress-induced changes in immune function in animals: Hypothalamo-pituitary-adrenal influences. In R. Ader, D.L. Felten, & N. Cohen (eds.), *Psychoneuroimmunology*, pp. 771-784. Academic Press Inc., San Diego, California.

Klein, T. W., Newton, C. A., & Friedman, H. (1988). Suppression of human and mouse lymphocyte proliferation by cocaine. In T.P. Bridge (ed.), *Psychological, Neuropsychiatric, and Substance Abuse Aspects of Aids*, pp. 139-143. Raven Press: New York.

Lewis, J. W., Shavit, Y., Terman, G. W., Nelson, L. R., Gale, R. P., & Liebeskind, J. C. (1983). Apparent involvement of opioid peptides in stress-induced enhancement of tumor growth. *Peptides*, **4**, 635-638.

Lysle, D. T., Lyte, M., Fowler, H., & Rabin, B.S. (1987). Shock-induced modulation of lymphocyte reactivity: Suppression, habituation and recovery. *Life Sciences*, **41**, 1805-1814.

Mokler, D.J., Correla, K.M., & Novotny, J.F. (1991). Effect of chronic cocaine on the behavioral and immunological effects of chronic stress in mice. *NIDA Research Monograph Series*, **105**, 543-544.

Monjan, A.A., & Collector, M.I. (1976). Stress-induced modulation of the immune response. *Science*, **196**, 307-308.

Pezzone, M.A., Rush, K.A., Kusnecov, A.W., Wood, P.G., & Rabin, B.S. (1992). Corticosterone-independent alteration of lymphocyte mitogenic function by amphetamine. *Brain Behavior and Immunity*, **6**, 293-299.

Raygada, M., Shaham, Y., Nespor, S.M., Kant, G.J., & Grunberg, N.E. (1992). Effects of stress on hypothalamic insulin in rats. *Brain Research Bulletin*, **29**, 129-134.

Raygada, M., & Baum, A. (1991). Aids, drug use, and psychopharmacology. *Psychoparmacology Newsletter*, **23**, 2-6.

Shavit, Y., & Martin, F. C. (1987). Opiates, stress, and immunity: Animal Studies. *Annals of Behavioral Medicine*, **5**, 11-15.

Strausbaugh, H. & Irwin, M. (1992). Central corticotropin-releasing hormone reduces cellular immunity. *Brain, Behavior and Immunity*, **6**, 11-17.

Van Dyke, C., Stesin, A., Jones, R., Chuntharapai, A., & Seaman, W. (1986). Cocaine increase,s natural killer cell activity. *The Journal of Clinical Investigation*, **77**, 1387-1390.

Wainer, H. (1982). Robust statistics: A survey and some prescriptions. In G. Keren (ed.), *Statistical and Methodological Issues in Psychology and Social Sciences Research*, pp. 70-95. Lawrence Earlbaum Associates, Hillsdale, N.J.

Watson, E. S., Murphy, J. C., Elsohly, H. N., Elsohly, M. A., & Turner, C. E. (1983). Effects of the administration of coca alkaloids on the primary immune responses of mice: Interaction with D9-tetrahydrocannabinol and ethanol. *Toxicology and Applied Pharmacology*, **71**, 1-13.

Weise, R.D., & Kopin, I.J. (1976). Assay of catecholamines in human plasma: studies of a singleisotope radioenzymatic procedure. *Life Sciences*, **19**, 1673-686.

Wills, T.A. (1986). Stress and coping in early adolesence: Relationships to substance use in urban school samples. *Health Psychology*, **5**(6), 503-529.