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Infectious Diseases and Substance Abuse

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Preface to the Series

The mechanisms of disease production by infectious agents are presently the focus of an unprecedented flowering of studies. The field has undoubtedly received impetus from the considerable advances recently made in the understanding of the structure, biochemistry, and biology of viruses, bacteria, fungi, and other parasites. Another contributing factor is our improved knowledge of immune responses and other adaptive or constitutive mechanisms by which hosts react to infection. Furthermore, recombinant DNA technology, monoclonal antibodies, and other newer methodologies have provided the technical tools for examining questions previously considered too complex to be successfully tackled. The most important incentive of all is probably the regenerated idea that infection might be the initiating event in many clinical entities presently classified as idiopathic or of uncertain origin.

Infectious pathogenesis research holds great promise. As more information is uncovered, it is becoming increasingly apparent that our present knowledge of the pathogenic potential of agents is often limited to the most noticeable effects, which sometimes represent only the tip of the iceberg. For example, it is now well appreciated that pathologic processes caused by infectious agents may emerge clinically after an incubation of decades and may result from genetic, immunologic, and other indirect routes more than from the infecting agent in itself. Thus, there is a general expectation that continued investigation will lead to the isolation of new agents of infection, the identification of hitherto unsuspected etiologic correlations, and, eventually, more effective approaches to prevention and therapy.

Studies on the mechanisms of disease caused by infectious agents demand a breadth of understanding across many specialized areas, as well as much cooperation between clinicians and experimentalists. The series *Infectious Agents and Pathogenesis* is intended not only to document the state of the art in this fascinating and challenging field but also to help lay bridges among diverse areas and people.

Mauro Bendinelli
Herman Friedman

Foreword and Introduction

The use of recreational drugs of abuse by large numbers of individuals in this country and abroad has aroused serious concerns about the consequences of this activity. For example, it is recognized that marijuana is currently widely used as a recreational drug in the United States as well as other countries. Similarly, abuse of cocaine, especially crack cocaine, is considered to be an epidemic. "The war on drugs" by the US Government was directly aimed at the illicit use of cocaine, marijuana, and opiates as well as other drugs of abuse. Furthermore, alcohol is also considered a major problem of abuse in this country as well as in many other countries. It is estimated there are at least 10 million alcoholics in the United States alone. A significant portion of those hospitalized with infectious diseases are alcoholics. Similarly, there have been many reports of association between marijuana use and increased susceptibility to infection as well as a relation between use of opiates and infections. The relationship between drug abuse and increased incidence of various infections has stimulated increased investigation of whether and how such drugs affect immune function, especially important for resistance against infectious agents.

During the last decades, a wide variety of studies have shown that drugs of abuse, including marijuana, cocaine, or opiates, as well as alcohol, alter both neurophysiological as well as pathological responses of individuals. Similarly, it has been shown that illicit drug use also alters immune function, and the influence of such altered immunity has marked physiological and physical consequences on drug abusers. Specifically, data have accumulated indicating that drugs of abuse markedly affect the immune response in both human populations and in experimental animal models, both *in vivo* and *in vitro*.

Experimental studies concerning microbial infections in animals have supported empirical observations reported earlier that many drugs of abuse are often associated with enhanced susceptibility to infectious diseases. Furthermore, the mechanisms whereby such drugs increase the likelihood of infections in humans as well as experimental animals have begun to be delineated. In particular, it has been shown that morphine, cocaine, or marijuana, as well as alcohol, enhance susceptibility to infection by bacteria, viruses, protozoa, or fungi when given to experimental animals or used to treat lymphoid cell

populations *in vitro*. The purpose of this volume is to focus attention on valuable new information concerning the effects of recreational drugs on modulation of immune responses, especially pertaining to mechanisms important in resistance to infectious diseases, as well as to malignancy and autoimmunity. Studies concerning how illicit drugs affect immunity are considered even more urgent at the present time because of the worldwide epidemic of acquired immune deficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV). Infection with HIV causes the collapse of the immune system, making an individual highly susceptible to opportunistic microorganisms which cause significant clinical disease in mainly immunocompromised individuals.

The onset of the AIDS epidemic in the United States, and indeed worldwide, stimulated attempts to search for possible cofactors which result in a more rapid progression of the disease in individuals infected with HIV. Approximately a third of all AIDS patients in the United States and other developed countries are i.v. drug abusers. It has been shown that HIV is readily spread by contaminated needles or equipment used by drug abusers. However, it is also widely accepted that many illicit drugs not taken by the i.v. route but by other routes are immunosuppressive and modulate the immune system, especially by activation hypothalamic–pituitary–adrenal axis. Although many AIDS patients, especially in third world countries, are known not to be i.v. drug abusers, they often utilize drugs such as marijuana, cocaine, or even alcohol, and HIV may be transmitted by the sexual route, even in such drug abusers. Thus, there is much concern that such illicit drugs serve as a possible cofactor in the progression of AIDS.

There had been various studies during the past few years examining in detail the mechanisms whereby drugs of abuse compromise the immune system in general and specifically enhance susceptibility to infection. Thus, this book in the series *Infectious Agents and Pathogenesis* focuses specifically on possible relationships between drugs of abuse like cocaine, marijuana, and opiates, as well as alcohol, immune response function, and alteration of resistance to microorganisms, especially opportunistic bacteria. This volume presents a number of reviews concerning various categories of drugs, the immune system, and infectious disease. The first chapter is a detailed review by investigators from Georgetown University concerning the effects of both cocaine and morphine in animal models with regard to the nature and mechanism of immunomodulation resulting from acute withdrawal. The next chapter is by Drs. Baldwin and Roth from UCLA concerning links between cannabinoid use and HIV infection. Drs. Bulen and Medveczky from the University of South Florida then discuss the effects of cannabinoids on Herpesvirus infection and the mechanisms involved.

Drs. Guy Cabral and Francine Marciano-Cabral from the Medical College of Virginia describe studies concerning the effects of cannabinoids on increased susceptibility of brain cells to infection by an important amoeba known to cause neurologic disease. Investigations concerning nature and mechanisms whereby cannabinoids specifically alter susceptibility to infection by the ubiquitous opportunistic intracellular microbe *Legionella pneumophila* are then described in detail in the following chapter. Nicotine is now recognized as the addictive component of cigarette smoke and the next seven chapters review in detail studies concerning how nicotine affects the immune response, especially those aspects

of immunity important in host resistance. It is widely recognized that cigarette smokers are more susceptible to upper respiratory infections by bacteria or viruses.

The next several chapters concern the effects of opiates on the immune system. Investigators from Temple University in Philadelphia describe studies concerning the effect of opiates on regulation of chemokine and chemokine receptor expression, known to be important in host resistance mechanisms, especially with emphasis on HIV infection. The next chapter by Dr. Roy and associates from Minnesota describes the effects of morphine on immune response mechanisms important in susceptibility to infections. Dr. Sharp and colleagues from Tennessee describe studies concerning neuropharmacological aspects of delta opioid receptors on murine splenic T cells and involvement of these receptors in immunity. Investigators from the University of Illinois then describe some of the effects of opiate derivatives on immunity, especially as related to mechanisms of resistance to infectious agents. The next several chapters discuss different aspects of the effects of alcohol on immunity, especially susceptibility to opportunistic bacteria and fungal infection. An experimental animal model is described concerning opportunistic infection by *Brucella* and ethanol. A general description of effects of alcohol on respiratory infections and the pulmonary system is then presented.

It is anticipated by the editors of this volume and the series in general, as well as the authors of individual chapters, that this book will be valuable for microbiologists, both basic and clinical, as well as immunologists, psychologists, and drug abuse investigators, including health care workers who care for and rehabilitate drug abusers. It is also anticipated that this book will also provide important information concerning the public health impact of drugs of abuse on infectious diseases. It is also hoped by the editors that the information presented will stimulate further interest and studies concerning the effects of drugs of abuse on infectious diseases. The editors thank Ms. Ilona M. Friedman for continued outstanding contributions as the coordinator for preparation of this volume and for valuable assistance in processing and editing manuscripts for publication.

Herman Friedman
Thomas W. Klein
Mauro Bendinelli

Contents

1

Effects of Cocaine and Morphine Withdrawal on the Immune Response

ALBERT H. AVILA, NORMA C. ALONZO, and BARBARA M. BAYER

1. INTRODUCTION

The immunosuppression accompanying illicit drug use has been shown to contribute to a decreased resistance to a variety of pathogens; however, there is relatively little information on how long these effects persist following withdrawal from chronic drug exposure. To begin to address this question, Sprague–Dawley male rats were administered either cocaine (10 mg/kg, i.p., b.i.d.) for 7 days or morphine (escalating doses up to 40 mg/kg, s.c., b.i.d.) for a 10-day period. Control groups of animals received similar saline injections for equivalent time periods. Drug administration was abruptly discontinued and animals were sacrificed at 2, 24, 72, or 96 hr following the last dose. At these time points, proliferation responses of peripheral blood T lymphocytes stimulated by concanavalin A (Con A) and plasma levels of corticosterone were measured. Plasma corticosterone levels of cocaine- or morphine-treated animals were found to be significantly elevated 24 hr following drug cessation as compared to saline-treated animals. At this time, proliferation responses were significantly decreased and were further suppressed during cocaine and morphine withdrawal at 96 and 72 hr, respectively. These results suggest that abrupt cessation of cocaine or morphine administration leads to activation of stress-related

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pathways that may contribute to an increased susceptibility of infection during the initial withdrawal phase.

It is well known that cocaine and morphine abuse in general is a major health concern in our society. Studies have shown a high risk factor related to HIV seropositivity among cocaine users.[1,2] There appears to be an association between drug abuse populations and the development of AIDS, thus leading to the belief that the use of such drugs may serve as a cofactor in the pathogenesis of AIDS.[3,4] However, it is not clear if the immune alterations and susceptibility to AIDS is due to the lifestyle of the drug user (needles, nutrition, sexual practices) or to the effects of the drug itself.

Chronic cocaine and morphine exposure has been likened to the stress response due to their effect on the hypothalamic–pituitary–adrenal (HPA) axis resulting in elevation in plasma glucocorticoid levels.[5,6] As a result, many laboratories have investigated the potential interaction between HPA axis activation, stress and drug addiction, or relapse.[7–9] It is known that prolonged and permanent alterations within the central nervous system (CNS) occur following chronic cocaine or morphine administration,[6,10–12] as well as following withdrawal from either drug.[13–15] In addition to chronic exposure, abrupt withdrawal from chronic cocaine[16,17] or morphine[18] has also been shown to produce neuroendocrine alterations. Many of these effects have been thought to contribute to the immune deficiencies that accompany acute and chronic exposure to these drugs.[19–22] However, little is known of the potential impact that withdrawal from either morphine or cocaine has on the immune system. This is particularly surprising considering reports that cocaine abuse and dependence remains a major public health problem.[4] This is also surprising because many cocaine and drug abusers have a high potential for HIV exposure. If the immune system is compromised during the time of HIV exposure, the likelihood of higher viral titers and the susceptibility to contracting the disease is increased. Therefore, in this chapter, we begin to define the effects during cocaine or morphine exposure as well as during the early stages of withdrawal from chronic cocaine or morphine exposure on both the HPA axis and the immune system.

2. MATERIALS AND METHODS

2.1. Animals

Pathogen-free adult male Sprague–Dawley rats initially weighing 175–200 g upon receipt were obtained from Taconic Laboratories (Germantown, NY). Animals were group-housed, three per cage, with microisolator tops in a temperature (23 ± 1°C) and humidity-controlled vivarium under a 12-hr light/dark cycle (6 AM on, 6 PM off). Food and water were freely available (Purina rat chow, Ralston Purina Co., St. Louis, MO). All animals were allowed to acclimate for 1 week before use in an experiment or drug administration.

2.2. Drug Administration

Cocaine hydrochloride, purchased from Sigma Chemical (St. Louis, MO), and morphine sulfate, generously provided by the National Institute on Drug Abuse (Research Triangle Park, NC) were dissolved in (0.9%) sterile isotonic saline, which also served as the control treatment in these studies. The injection volume for both cocaine and morphine studies was 1 ml/kg and the route of administration was intraperitoneal (i.p.) for cocaine injections, and subcutaneous (s.c.) for morphine injections. For all cocaine injections, the rats received 10 mg/kg for 7 days (b.i.d.). For morphine injections, the animals were given escalating doses of morphine from 10 to 40 mg/kg for 9 days (b.i.d.), and were challenged with a 10 mg/kg injection of morphine on day 10. Animals were sacrificed 2 hr following the last cocaine injection or following respective withdrawal periods (24, 72, or 96 hr).

2.3. Mitogen-Induced Lymphocyte Proliferation

Rats were sacrificed by rapid decapitation, and trunk blood was collected in 50-ml polypropylene tubes containing heparin (0.1 ml) and immediately placed on ice. Whole blood was diluted 1:5 with cold RPMI-1640 media (Gibco BRL/Life Technologies, Grand Island, NY) containing 1% fetal calf serum and gentamicin (20 g/ml). Hundred liters of each blood suspension was plated into 96-well flat-bottom microtiter plates containing nine concentrations of the T-cell-specific mitogen Con A (100 L/well), incubated for 72 hr at 37°C with 8% CO_2 and pulsed with 0.5 Ci/well of [methyl-^{3}H]thymidine (6.7 Ci/mmol; New England Nuclear, Boston, MA) in a 20 L volume followed by additional 24 hr incubation. Cells were lysed by distilled water using a 96-well cell harvester (Brandel, Gaithersburg, MD), and labeled DNA was harvested onto glass fiber filters. Radioactivity was quantified via liquid scintillation spectrophotometry (Beta Plate; L.K.B. Pharmacia). Maximum lymphocyte proliferation responses (E_{max}) were determined from a nonlinear regression analysis of T-cell response to the mitogen Con A, and significant differences in E_{max} values were assessed using one-way ANOVA and Newman Keuls *post hoc* analysis.

2.4. Plasma Corticosterone Assay

Heparinized blood samples were collected at the time of sacrifice, placed on ice, and centrifuged to allow separation of plasma that was collected and stored at −20°C until needed. Plasma corticosterone was measured using solid-phase double antibody ^{125}I radioimmunoassay kits purchased from ICN Biochemicals, Inc. (Costa Mesa, CA). Samples were assayed in duplicate, and corticosterone concentrations were expressed as nanograms per milliliter.

3. RESULTS

3.1. Immune and HPA Axis Effects from Acute Cocaine or Acute Morphine

As an initial assessment of cocaine's effects on the immune system, rats were injected with either acute cocaine (10 mg/kg, i.p.) or acute morphine (10 mg/kg, s.c.) and compared with saline control animals. All animals were sacrificed 2 hr following the injection, and blood was stimulated with increasing doses of the T-cell mitogen Con A. Maximum responses (E_{max}) were determined from a nonlinear regression analysis utilizing all concentrations of Con A, and significant differences in the E_{max} values were determined using the Student's *t*-test. T-lymphocyte proliferation did not differ between acute cocaine- and saline-treated animals (Fig. 1). In contrast to acute cocaine, acute morphine resulted in a significant suppression of blood lymphocyte proliferation ($p < 0.05$) (Fig. 2).

It is known that drugs of abuse can have stress-like effects on the HPA axis. To determine if there were any neuroendocrine effects, plasma corticosterone levels were measured in all animals. Animals were treated with either acute cocaine (10 mg/kg, i.p.) or saline and sacrificed 2 hr later. There were no significant differences in corticosterone levels at 2 hr between acute cocaine and saline control animals (Fig. 3). In contrast to cocaine, acute morphine (10 mg/kg, i.p.) led to a significant increase in plasma corticosterone levels 2 hr after a single morphine injection ($p < 0.05$) (Fig. 4).

3.2. Immune Effects Following Chronic Cocaine or Morphine

To determine whether chronic exposure to cocaine had effects on the immune system, animals were exposed to cocaine (10 mg/kg, i.p., b.i.p.) for 7 days. All animals were sacrificed 2 hr after the final injection. There was a

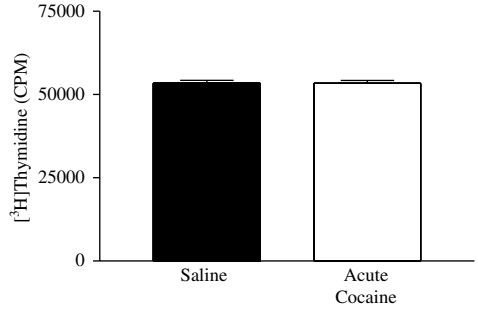

FIGURE 1. Effect of acute cocaine administration on blood lymphocyte proliferation. Animals (6 per group) were injected with cocaine (10 mg/kg, i.p.) or saline and sacrificed 2 hr following injection. Blood was collected into heparinized tubes, diluted 1:5 and lymphocyte proliferation stimulated by Con A. Data are expressed as E_{max} [^{3}H]methyl-thymidine ± SEM. No significant difference in E_{max} values ($p > 0.05$, *t*-test).

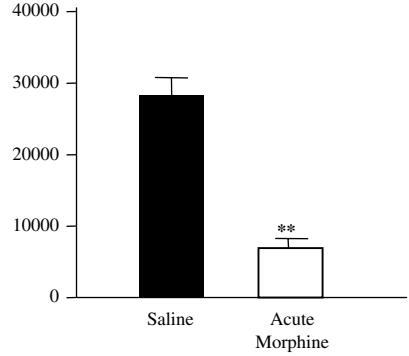

FIGURE 2. Effect of acute morphine administration on blood lymphocyte proliferation. Animals (8 per group) were injected with morphine (10 mg/kg, s.c.) or saline and sacrificed 2 hr following injection. Blood was collected into heparinized tubes, diluted 1:5 and lymphocyte proliferation stimulated by Con A. Data are expressed as E_{max} [^{3}H]methyl-thymidine ± SEM. Significant difference in E_{max} values ($p < 0.05$, *t*-test).

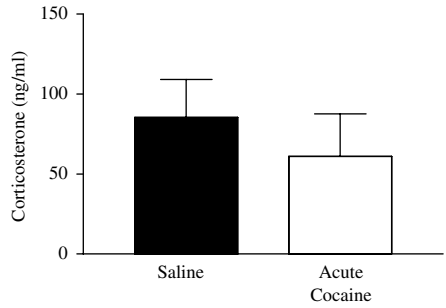

FIGURE 3. Effect of acute cocaine administration on plasma corticosterone. Animals (6 per group) were treated with either a single injection of cocaine (10 mg/kg, i.p.) or saline (1 ml/kg) and sacrificed 2 hr after injection via decapitation. Plasma corticosterone levels were determined as described in methods and expressed as mean (ng/ml) ± SEM. No significant alteration was detected ($P > 0.05$, Student's *t*-test).

significant decreased in T-cell proliferation in chronic cocaine animals compared to similarly treated saline controls (Fig. 5). Interestingly, following cessation of drug administration, this effect persisted for up to 96 hr following the last dose of cocaine ($p < 0.05$). Furthermore, animals that underwent 96 hr of withdrawal from cocaine were statistically more suppressed than those of the chronic cocaine group or the animals undergoing 24 hr of withdrawal.

Unlike chronic cocaine, chronic morphine treatment resulted in a tolerance to the suppressive effects of morphine on T-lymphocyte proliferation (Fig. 6). However, a significant suppression of lymphocyte responses developed within 24 hr after cessation of chronic morphine administration. The suppression of lymphocyte proliferation was significant for up to 72 hr of withdrawal from chronic morphine (Fig. 6).