Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 483 (2004) 309–315
www.elsevier.com/locate/ejphar

The effect of cocaine sensitization on mouse immunoreactivity

Marta Kubera[a,*], Małgorzata Filip[b], Agnieszka Basta-Kaim[a], Ewa Nowak[b], Joanna Siwanowicz[b], Alena Zajicova[c], Vladimir Holan[c], Michael Maes[d,e,f], Władysław Lasoń[a]

[a] *Department of Endocrinology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland*
[b] *Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland*
[c] *Institute of Molecular Genetics, Academy of Sciences, Flemingovo nam. 2, 166 37 Prague 6, Czech Republic*
[d] *Department of Psychiatry and Neuropsychology, University of Maastricht, Maastricht, The Netherlands*
[e] *Clinical Research Center for Mental Health, Limburg, Belgium*
[f] *Department of Psychiatry, Vanderbilt University, Nashville, TN, USA*

Received 23 June 2003; received in revised form 14 October 2003; accepted 17 October 2003

Abstract

Recent studies indicate a role of the immune system in the behavioral effects of cocaine in rodents. In the present study, we attempted to find a correlation between the behavioral changes induced by repeated, intermittent administration of cocaine and some immunological consequences of sensitization to cocaine. Male Albino Swiss mice were treated repeatedly (for 5 days) with cocaine (10 or 15 mg/kg, intraperitoneally, *ip*). On day 9, they received a challenge dose of cocaine (10 or 15 mg/kg). Acute administration of cocaine increased the locomotor activity of mice. In animals treated repeatedly with the higher dose of cocaine, the locomotor hyperactivity induced by a challenge dose of the psychostimulant (15 mg/kg) was ca. twice as high as that after its first administration; in consequence, evidence for behavioral sensitization was obtained. Immune functions were evaluated by measuring the ability of splenocytes to proliferate and to produce cytokines such as interferon-γ (IFN-γ), interleukin (IL)-4 and IL-10. Acute cocaine administration significantly decreased proliferation of splenocytes to concanavalin A (Con A) and increased their ability to produce IFN-γ. Repeated intermittent treatment with cocaine in a dose of 10 mg/kg significantly decreased the thymus weight and the proliferative response of T cells to a suboptimal dose of Con A. Sensitization with the higher dose of cocaine significantly enhanced IFN-γ production. These data indicate that cocaine sensitization results in the development of a tolerant state to the cocaine-induced suppression of a thymus dependent T-lymphocyte response. It may be suggested that the cocaine sensitization partly depends on the altered balance of cytokine production, e.g. an increase in IFN-γ production. Since repeated, intermittent use of cocaine by humans leads to psychoses or craving for this drug, our findings also seem to indicate considerable importance of monitoring and correcting immune changes in the therapy of cocaine addiction.

Keywords: Cocaine sensitization; Locomotor hyperactivity; Immunity; Cytokine; Splenocyte proliferation; Interferon-γ

1. Introduction

Efforts have been made to understand the mechanism of the behavioral effects produced by acute or chronic treatment with cocaine. Acute injection of this psychostimulant to rodents produces—among other things—locomotor hyperactivity, which may mimic mania with hyperexcitable effects in humans (Kalivas et al., 1998). A repeated, intermittent regimen of cocaine injections augments the behavioral response (e.g. locomotor effects) to a challenge dose (the same or lower) of the psychostimulant following its re-administration (Robinson and Berridge, 1993). The latter phenomenon, called sensitization, is long-lasting and seems to be closely related to cocaine addiction and drug-dependent paranoid psychoses (Robinson and Berridge, 1993; Kalivas et al., 1998). Moreover, discontinuation of repeated cocaine use may produce a withdrawal syndrome with craving for the drug and symptoms resembling endogenous depression (Kalivas et al., 1998).

On the other hand, the incidence of infectious diseases and immune system dysfunctions is raised among individuals in a drug-abusing population. It has been shown that cocaine may serve as a co-factor in the development of

* Corresponding author. Tel.: +48-12-662-3273; fax: +48-12-637-4500.
E-mail address: kubera@if-pan.krakow.pl (M. Kubera).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.10.021

acquired immune deficiency syndrome (AIDS) by increasing susceptibility to retrovirus infection (Chaisson et al., 1989; Weiss, 1989). Apart from the above, other data also suggest that cocaine induces immune abnormalities (Pellegrino and Bayer, 1998). Thus, cocaine has been found to produce general immunosuppressive effects in vitro and contradictory effects after its administration to humans and animals. In fact, the in vivo studies have demonstrated that cocaine produces either inhibition, or enhancement, or has no effect on several aspects of the immune response, such as the number and activity of natural killer (NK) cells, the ability of splenocytes to proliferate or to produce cytokines after mitogen stimulation (Van Dyke et al., 1986; Di Francesco et al., 1991; Ruiz et al., 1994; Pellegrino and Bayer, 1998). Such discrepancies in the effect of cocaine on the immune system may reflect differences in the dosage regimens and in the duration of exposure to cocaine. To the best of our knowledge, cocaine-induced behavioral changes have never been correlated with its effect on the immune system. Correlation of the behavioral changes with the immune response is a useful approach to study the mechanism of drug actions. For example, our previous investigations of immunoreactivity in animal models of depression have brought support to the concept that the therapeutic activity of antidepressant drugs is connected with their effect not only on the central nervous system but also on the immune system, as reflected by inhibition of depressogenic cytokine production and stimulation production of interleukin (IL)-10, a negative immunoregulatory cytokine (Kubera et al., 1996; Kubera et al., 2001).

In the present paper, we described the effects of repeated, intermittent administration of cocaine in sensitizing and non-sensitizing doses on some parameters of immune system activity. Animals were treated repeatedly (for 5 days) with cocaine; afterwards, on day 9, they received a challenge dose of cocaine. Since sensitization to cocaine is related to the dose of the drug and conditioning (specific environmental cues associated with drug treatment) and the withdrawal time (Pierce and Kalivas, 1997), we examined two different doses of cocaine (10 and 15 mg/kg) under conditions of minimal influence of the environment on the process of sensitization and in a short withdrawal period. Cocaine sensitization was estimated by locomotor activity measurements (Przegalinski et al., 2000).

In cocaine-treated mice, the weight of the spleen and thymus, the proliferative and metabolic activity of splenocytes, and the ability of splenocytes to produce IL-4, IL-10 and interferon-gamma (IFN-γ) were studied. The thymus plays a pivotal role in T-cell development, whereas the spleen is involved in T- and B-cell-dependent immune responses. The drug-induced changes in the weight of these two organs may also reflect alterations in thymocyte development and splenocyte activity. The balance between cellular and humoral immune responses to antigens is controlled via communication between immunocompetent cells. The responses are regulated to a great extent by cytokines which are produced predominantly by T helper (Th) cells and macrophages. Cytokines are biologically active polypeptide intracellular messengers which regulate the growth, mobility, and differentiation of leukocytes. IL-4 has initially been described as a co-stimulator of proliferation of B cells. It also acts as a growth factor for T cells, and may influence them by inducing expression of the high affinity IL-2 receptor and production of IL-2. Moreover, IL-4 has been shown to induce macrophages to express MHC molecules and to activate them for the purpose of killing tumour cells (Le Gros et al., 1990). IL-10 inhibits the production of monokines, as well as the differentiation and production of cytokines by Th1 cells (Scott and Kaufmann, 1991). IFN-γ is involved in multiple biological responses including an antiviral activity, activation of the phagocytosis of macrophages, cytotoxicity of NK cells, augmentation the development of Th1 cells, inhibition of the differentiation of Th2 cells and of the production of Th2 cytokines (Gajewski et al., 1989).

2. Materials and methods

2.1. Animals

The experiment was performed on male Albino-Swiss mice (about 12 weeks old at the start of the experiment). The animals were housed in groups of 10 per cage and were kept at a room temperature of 20 ± 1 °C on a 12-h light/dark cycle (the light was on between 06.00 and 18.00 h). The mice had free access to food and water. All the tests were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Kraków which met the International Guide for the Care and Use of Laboratory Animals.

2.2. Drug

Cocaine hydrochloride (Sigma, USA) was used. Cocaine was dissolved in saline and administered intraperitoneally (*ip*) in a volume of 0.1 ml/10 g.

2.3. Locomotor activity measurement

The locomotor activity of mice was recorded individually in photoresistor actometers on days 1, 5 and 9 for each animal, as described previously (Chojnacka-Wójcik, 1992). Measurement of the animals' activity started 5 min after drug injection (see below). Locomotor activity was recorded for 60 min. Ten animals per group were used.

2.4. Sensitization protocol

During the first 5 days of the experiment, the animals received the following injections: vehicle and cocaine, 10 or

15 mg/kg. On day 9, the mice were challenged with cocaine (10 or 15 mg/kg) or vehicle (control group).

2.5. Preparation of cell suspensions

For immunological studies, the animals were sacrificed 1 h after the last injection and their spleens and thymuses were gently removed and weighed. The spleens were crushed in individual glass homogenizers, dipped in ice. The obtained cells were suspended in RPMI-1640 medium (Sigma), and were centrifuged at $500 \times g$ for 5 min. Cell pellets were resuspended in the same medium for a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) assay, or in the same medium supplemented with antibiotics and a 10% fetal bovine serum (Sigma) for other studies.

2.6. Colorimetric MTT assay

The MTT assay is based on the conversion of a yellow tetrazolium salt to a coloured formazan product by enzymes in viable cells. This assay is widely used in cell proliferation and cytotoxicity studies. The cellular bioreduction of MTT is associated with mitochondrial dehydrogenases and with enzymes of the endoplasmatic reticulum; it involves the reduced pyridine nucleotides NADH and—to a lesser extent—NADPH. The concentration of the coloured product can be measured spectrophotometrically and is proportional to the number of viable cells. This method was described by us previously (Kubera et al., 1998). In short, MTT (Sigma) was dissolved in PBS at 5 mg/ml. A stock MTT solution (10/100 μl of splenocytes; a suspension of 4×10^6 splenocytes per ml) was added to all the wells, and the 96-well plates were incubated at 37 °C for 1.5 h. A hundred microliters of lysis buffer (acid-isopropanol; POCh, Poland) was added to each well, and the plates were read 30 min later with a Uniscan II reader (Labsystem, Finland) at a wavelength of 570 nm.

2.7. Proliferative activity of splenocytes

The proliferative response of spleen cells was described earlier by Kubera et al. (1995). Briefly, 2×10^5 cells per well were stimulated with concanavalin A (Con A; 0.6 and 1.25 μg/ml), phytohemagglutinin (PHA-P; 5 and 10 μg/ml) and lipopolysaccharide (LPS; 1.25 μg/ml) and were incubated in 96-well plates at 37 °C at a final volume of 0.2 ml for 72 h. Cell proliferation was determined by adding 0.5 μCi of [^{3}H]-thymidine per well (ICN, USA; SpA 6.7 Ci/mmol) at 16 h before the end of the incubation.

2.8. Lymphokine detection and quantification

Splenocytes were tested for their ability to produce IL-4, IL-10 and IFN-γ after stimulation with Con A. Suspensions of the splenocytes were seeded at a concentration of 2×10^6 cells/ml in 24-well corning tissue culture plates and were then stimulated with a Con A solution (1.25 μg/ml). Cell-free supernatants were collected 48 h later for IFN-γ or 72 h later for IL-4 and IL-10. The supernatants were stored at − 20 °C. In preliminary studies, in the splenocyte cultures stimulated with Con A, the highest level of IFN-γ was observed in 48-h culture supernatants and those of IL-4 and IL-10 in 72-h ones.

Enzyme-linked immunosorbent assays (ELISA) was carried out as described earlier (Kubera et al., 2001). All the standards, i.e. a recombinant mouse IL-4 (mr IL-4, SpA 1×10^7 IU/ml), a recombinant mouse IL-10 (mr IL-10, SpA 5×10^6 IU/ml) or a recombinant mouse IFN-γ (mr IFN-γ, SpA $4.9–9.0 \times 10^6$ IU/ml), were purchased from Genzyme Diagnostics (USA).

2.9. Statistical analysis

Behavioral studies were carried out on five groups of animals. To evaluate behavioral sensitization, the response to cocaine on day 9 was compared with that to the first injection of cocaine (day 1) to the same animal, or with the response to test drug injection (day 9) to animals treated with repeated vehicle, using a paired Student *t*-test or a one-way analysis of variance (ANOVA), respectively. The one-way ANOVA, followed by post hoc Dunnett's test, were applied to evaluate the effect of the treatment group separately on days 1 and 9.

The thymus and spleen weight was estimated for four groups of animals. The group of animals repeatedly treated

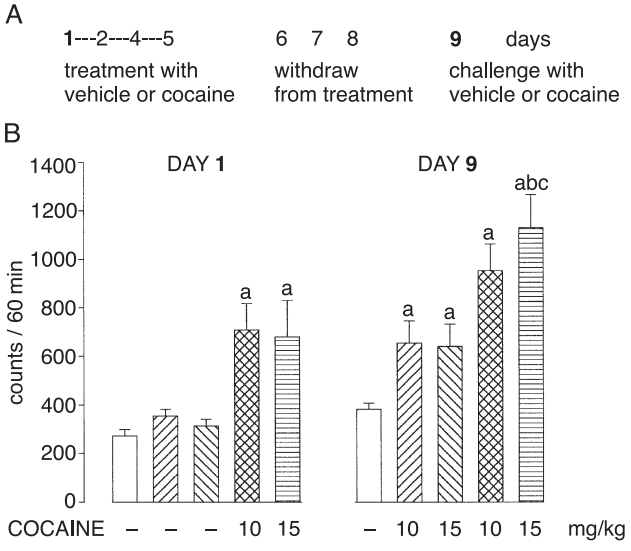

Fig. 1. Behavioral experiments with acute and repeated cocaine treatment. (A) Scheme of the experiment. (B) Locomotor activity data. Mice were treated repeatedly with vehicle, cocaine (10 or 15 mg/kg) daily for 5 days; on day 9, they were given a challenge dose of cocaine (10 or 15 mg/kg). Data are expressed as means ± S.E.M. $^aP < 0.001$ vs. corresponding vehicle controls; $^bP < 0.01$ vs. acute (15 mg/kg) cocaine group; $^cP < 0.01$ ($t = 4.13$) Student's paired *t*-test showed a significance between the effect of cocaine 15 mg/kg, on days 1 and 9; ANOVA showed a significant treatment group effect on days 1 [$F(4,44) = 3.99$, $P < 0.05$] and 9 [$F(4,44) = 4.56$, $P < 0.01$].

Table 1
The effect of cocaine administration on relative thymus and spleen weights and on metabolic activity of splenocytes

Treatment		Relative weight[a]					Metabolic activity[b] of splenocytes
Days 1–5 (mg/kg)	Day 9 (mg/kg)	*n*	Thymus	*n*	Spleen	*n*	
Vehicle	Vehicle	8	1.80 ± 0.14	9	4.50 ± 0.5	5	42 ± 16
Vehicle	Cocaine 15	10	1.64 ± 0.42	10	5.27 ± 0.4	5	54 ± 9
Cocaine 15	Cocaine 15	10	1.54 ± 0.30	10	4.86 ± 0.14	5	82 ± 30[c]
Cocaine 10	Cocaine 10	10	1.33 ± 0.33[c]	10	5.15 ± 0.25	5	98 ± 16[c]

Data expressed as means ± S.D., *n* = number animals per group.
[a] The relative spleen or thymus weight was estimated as weight of spleen or thymus in mg divided by body weight in g.
[b] The metabolic activity of splenocytes (MTT assay) is expressed in optical density units at 570 nm. MTT assay for splenocytes was determined in quadruplicate for each animal 1 h after last injection.
[c] $P < 0.05$ vs. vehicle-treated control (Fisher′ s LSD).

with vehicle for the first 5 days and challenged with cocaine, 10 mg/kg, on day 9 was discarded from the immunological study due to the same results in behavioral measurements as observed after cocaine 15 mg/kg on day 9. For other immunological studies, five animals were randomly chosen from each group. A one-way ANOVA was employed to assess the differences in immune markers between the four conditions, i.e. vehicle, acute cocaine (15 mg/kg), repeated cocaine (15 and 10 mg/kg). If any significant change was found, post hoc comparisons were performed using Fisher's LSD.

3. Results

3.1. Behavioral studies

On day 1 of the experiment, cocaine, 10 or 15 mg/kg, induced a ca. two-fold increase in the locomotor activity of mice (Fig. 1).

On day 9 of the experiment, in mice treated repeatedly (days 1–5) with cocaine (10 mg/kg), the challenge dose of the drug (10 mg/kg) increased their locomotor hyperactivity by ca. 40% compared to the respective controls with the first injection of the psychostimulant (Fig. 1); however, that enhancement of locomotor activity was insignificant as compared to acute cocaine.

A challenge with the higher dose of cocaine (15 mg/kg) on day 9 induced significant increase in locomotor hyperactivity of compared to the effect of the first injection of the psychostimulant (on day 1 to cocaine-treated animals, or on day 9 to vehicle-treated ones).

3.2. Thymus and spleen weight

Table 1 shows a significant decrease, by ca. 26%, in the relative thymus weight (the weight of the thymus divided by the body weight) of mice repeatedly treated with cocaine, 10 mg/kg. Acute and repeated injection of cocaine in the higher dose (15 mg/kg) decreased the thymus weight respectively by 9% and 14%; however, those results were not statistically significant. No significant differences in the spleen weights were found in any of the four groups studied, although acute cocaine administration and sensitization with cocaine, 15 mg/kg, increased relative spleen weight about 17% and 14%, respectively.

3.3. Cell metabolism activity (MTT assay)

Repeated, but not acute, injection of cocaine (10 and 15 mg/kg) increased reduction of the tetrazolium salt to formazan in an MTT assay, in comparison with the vehicle-treated control (Table 1).

3.4. Mitogen reactivity

The proliferative activity of splenocytes in response to stimulation of T-cell mitogens (Con A and PHA-P) in the group of mice treated acutely with cocaine was lower than that in the vehicle-treated control. Repeated treatment with

Table 2
The effect of cocaine administration on proliferative activity of splenocytes

Treatment		Con A		PHA-P		LPS
Days 1–5 (mg/kg)	Day 9 (mg/kg)	0.6 μg/ml	1.25 μg/ml	5 μg/ml	10 μg/ml	1.25 μg/ml
Vehicle	Vehicle	15779 ± 9441	59647 ± 9939	10864 ± 7593	70366 ± 23326	23586 ± 13353
Vehicle	Cocaine 15	4118 ± 1360[a]	33455 ± 5061[a]	2514 ± 920[a]	51526 ± 21901	17265 ± 10577
Cocaine 15	Cocaine 15	11484 ± 9690	44511 ± 12383	9579 ± 8450	56394 ± 14317	13232 ± 7802
Cocaine 10	Cocaine 10	4471 ± 1340[a]	49004 ± 17105	6490 ± 2435	60978 ± 25080	17123 ± 4855

Data expressed in cpm as means ± S.D.; *n* = 5 for each group. The proliferative activity of splenocytes was determined in triplicate for each animal 1 h after last injection.
[a] $P < 0.05$ vs. vehicle treated control mice (Fisher′ s LSD).

Table 3
The effect of cocaine administration on cytokines production by splenocytes stimulated by Con A

Treatment		IFN-γ (ng/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
Days 1–5 (mg/kg)	Day 9 (mg/kg)			
Vehicle	Vehicle	458 ± 41	134 ± 56	198 ± 44
Vehicle	Cocaine 15	509 ± 10[a]	110 ± 61	199 ± 43
Cocaine 15	Cocaine 15	543 ± 40[a]	90 ± 48	202 ± 57
Cocaine 10	Cocaine 10	454 ± 24	103 ± 54	117 ± 52[a]

Data expressed as means ± S.D.; $n = 5$ for each group. The ability of splenocytes to produce cytokines was determined in duplicate for each animal 1 h after last injection.

[a] $P < 0.05$ vs. vehicle-treated control (Fisher′ s LSD).

the lower dose of cocaine significantly decreased the blastogenic response of splenocytes to a suboptimal dose of Con A (Table 2).

3.5. Lymphokine production by splenic lymphocytes

The production of IFN-γ was significantly increased after acute and repeated administration of cocaine in a dose of 15 mg/kg. Repeated cocaine administration in a dose of 10 mg/kg significantly reduced IL-10 production by more than 40% in comparison with the vehicle-treated control. The ability of splenocytes to produce IL-4 was not changed in cocaine-treated animals (Table 3).

4. Discussion

In mice treated for 5 days with cocaine in a dose of 15 mg/kg, the locomotor hyperactivity induced by the same challenge dose of the psychostimulant tested after a 4-day withdrawal was about twice as high as that after its first administration. This observation may be regarded as evidence for behavioral sensitization to the locomotor stimulant effect of cocaine (Pierce and Kalivas, 1997). It should be added that in our study, the lower dose of the psychostimulant (10 mg/kg) enhanced the locomotor response of animals treated repeatedly with cocaine (10 mg/kg) as compared to saline. However, there was no correlation between acute (day 1) and challenge (day 9) dose of cocaine in the same group of animals. Regarding our experimental protocol, it should be stressed that sensitization to cocaine (15 mg/kg) did develop: (1) at the minimal ability of the environment (actometers) paired with cocaine, and may be considered as context-independent (the mice were given cocaine injections in experimental cages on days 1 and 5 only), and (2) during a short withdrawal period (there was only a 4-day withdrawal between the last repeated treatment with cocaine and administration of its challenge dose).

The effect of acute, short-term and prolonged cocaine administration on the immunity of experimental animals was the subject of several studies. To the best of our knowledge, the effect of cocaine sensitization on immunoreactivity has not been reported so far.

The main immune findings of this paper are: (1) any cocaine administration reduces the weight of the thymus, however, only repeated injection of 10 mg/kg of cocaine significantly decreased it; (2) acute cocaine administration decreases the proliferative activity of splenocytes in response to T-cells mitogens; (3) cocaine sensitization produced by repeated injection of the higher dose of cocaine (15 mg/kg) increases IFN-γ production, whereas repeated injection of the lower dose of cocaine (10 mg/kg) decreases the production of IL-10, a potent negative immunoregulator.

Reduction of the thymus weight is considered to be the most consistent change produced by cocaine administration. Higher effectiveness of the lower dose of cocaine in decreasing immunoreactivity was also reported by other studies on the ability of animals to eliminate tumour cells. Previously it was shown that the high doses of cocaine had no effect on tumour growth, whereas low doses accelerated tumour growth and increased the death rate in mice, probably by decreasing the cytotoxic activity of T and natural killer cells (Havas et al., 1987). It was also demonstrated that cocaine administration (1 and 10 mg/kg/day) to mice for 7 consecutive days reduced the absolute number of circulating white blood cells and the relative number of cells stained as lymphocytes, polymorphonuclear cells and monocytes. Moreover, the observed effect was stronger for the lower than for the higher dose of cocaine (Di Francesco et al., 1994). On the other hand, the lack of significant reduction of the thymus weight after acute cocaine administration in our studies was probably due to the very short (1-h) gap between cocaine administration and the animal sacrifice. Ou et al. (1989) observed a significant dose-dependent decrease in the number of mouse thymocytes, but 96 h after acute administration of cocaine.

Another finding of the present paper was that acute administration of cocaine, 15 mg/kg, resulted in a potent decrease in the Con A-induced T-cells proliferation, whereas the same dose of cocaine given repeatedly was ineffective. In line with our observation, Bayer et al. (1995) described a significant decrease in T-cell proliferation after acute, but not chronic, cocaine or morphine administration. Data from our laboratory with another psychostimulant, amphetamine, show that both acute and chronic administration of that drug reduces the Con A-induced responses (Kubera et al., 2002). Therefore, it may be speculated that addictive substances, such as cocaine and morphine, share a common mechanism to modulate proliferation of T cells (Bayer et al., 1996; Pellegrino and Bayer, 1998).

Apparent tolerance to the inhibitory effects of cocaine on T-lymphocyte proliferation after repeated cocaine administration does not necessarily indicate that the immunity of these animals returns to normal. For example, their susceptibility to the immuno-suppressive effects of stress may be considerably higher than in control animals, as was found for morphine-treated rats (Bayer et al., 1996).

In the present study, we observed a tendency towards a decrease in B-cell proliferation in response to LPS in mice sensitized to cocaine. This finding is in line with the results of other studies which showed a decrease in B-cell activity following chronic administration of high doses of cocaine, e.g. a decrease in the number of splenic plaque-forming antibodies (PFA) to sheep red blood cells (SRBC), a drop in the number of antibodies to the polysaccharide antigen SSS-III, and diminution in the number of IgM plaque-forming cells (Watson et al., 1983; Bagasra and Forman, 1989; Shen et al., 1994).

Although used extensively as a convenient and rapid measure of viable cells, the MTT assay has not always correlated well with other measures of cell growth and viability. In the present study, moderate decrease in splenocytes proliferation after chronic cocaine administration, estimated by ^{3}H-thymidine incorporation, did not correlate with a significant increase in the splenocytes MTT assay (Table 1). MTT does not permeate into lipid membranes, hence, it is taken up by cells through endocytosis and, after reduction, transported to the cell surface (Liu et al., 1997). Chronic cocaine administration increases the endocytosis of MTT, the exocytosis of MTT formazan and cellular MTT reductase activity in splenocytes.

In our experiments, single cocaine administration to mice pretreated with five injections of saline increased IFN-γ production and had no effect on IL-4 or IL-10 production. Di Francesco et al. (1999) obtained similar results in mice immunized with the influenza virus. On the other hand, Stanulis et al. (1997) reported an increase in IL-4 and IL-10 production following acute cocaine administration, while IFN-γ production was found to be unaffected. That effect was compared with that of single corticosterone injection according to the hypothesis that cocaine affects immune functions through the release of corticosterone. In fact, corticosterone administration in low physiological doses produced the same changes in immunity as did acute cocaine injection (Stanulis et al., 1997). The discrepancies between our data and those reported by Stanulis et al. (1997) probably stem from the differences in the experimental conditions (pretreatment of animals with five saline injections prior to cocaine one).

In the present paper, cocaine sensitization produced by repeated administration of a dose of 15 mg/kg increased the Th1-dependent IFN-γ production, whereas repeated injection of cocaine in a dose of 10 mg/kg inhibited the Th2-dependent IL-10 production. Decreases in IL-10 production were also reported by Wang et al. (1994), whereas Chen and Watson (1991) described the fostered ability of Th1 cells to produce TNF-α and IL-2 after chronic cocaine administration. However, these authors, did not find any changes in IFN-γ production after chronic cocaine administration, and observed even a reduction in IFN-γ production in mice infected with murine AIDS before cocaine administration (Chen and Watson, 1991). Cocaine sensitization increased IFN-γ production, which supports previous study performed in cocaine-dependent humans after cocaine infusion (Gan et al., 1998).

It is proposed that psychosis and schizophrenia may have an immunologic or viral etiology related to raised level of monocyte cytokines and a lowered one of Th-1-like cytokines, and an augmented quantity of Th-2-like cytokines (Maes et al., 2000). In psychoses and positive symptoms of schizophrenia (delusions, hallucinations, bizarre behavior), increase in IFN-γ production and/or a decrease in the production of IL-10 (among others, a negative regulator of INF-g production) may play some role (Inglot et al., 1994). On the other hand, it was shown that experimental application of IFN-γ to animals induced significant changes in behavior (Weinberg et al., 1988). On the basis of these data, it is speculated that the increase in IFN-γ production in an animal model of psychosis, described in the present paper, may be partially responsible for the observed changes in behavior.

The mechanism of the immunomodulatory effect of cocaine may be connected with its direct effects on immune cells, or with its indirect mechanism(s) exerted through activation of autonomic nervous system or the hypothalamic–pituitary–adrenal (HPA) axis.

In the immunosuppressive effects of cocaine, its impact on the monoamine level, as well as its local anaesthetic activity may play a particularly significant role. Among others, it has been shown that peripheral administration of the local anaesthetic lidocaine (5 mg/kg) or several monoamine reuptake inhibitors produces immunosuppressive effects similar to these observed after cocaine administration (Pellegrino et al., 2001).

In summary, our results show that the cocaine-induced immunomodulation is a complex phenomenon with a range of enhancing and inhibitory mechanisms which depend on the dose and duration of drug administration, as well as on the specific immune functions studied.

References

Bagasra, O., Forman, L., 1989. Functional analysis of lymphocyte subpopulations in experimental cocaine abuse: I. Dose-dependent activation of lymphocyte subsets. Clin. Exp. Immunol. 77, 289–293.

Bayer, B.M., Mulroney, S.E., Hernandez, M.C., Ding, X.Z., 1995. Acute infusion of cocaine result in time and dose-dependent effects on lymphocyte responses and corticosterone secretion in rats. Immunopharmacology 29, 19–28.

Bayer, B.M., Hernandez, M.C., Ding, X.Z., 1996. Tolerance and cross-tolerance in the suppressive effects of cocaine and morphine on lymphocyte proliferation. Pharmacol. Biochem. Behav. 53, 227–234.

Chaisson, R.E., Bacchetti, P., Osmond, D., Brodie, B., Sande, M.A., Moss, A.R., 1989. Cocaine use and HIV infection in intravenous drug users in San Francisco. JAMA 261, 561–565.

Chen, G., Watson, R.R., 1991. Modulation of tumor necrosis factor and gamma-interferon production by cocaine and morphine in aging mice infected with LP-BM5, a murine retrovirus. J. Leukoc. Biol. 50, 349–355.

Chojnacka-Wójcik, E., 1992. Involvement of dopamine autoreceptors in the hypoactivity induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in mice. Pol. J. Pharmacol. Pharm. 44, 135–146.

Di Francesco, P.D., Pica, F., Marini, S., Favalli, C., Garaci, E., 1991. Thymosin alfa one restores murine T-cell mediated responses inhibited by in vivo cocaine administration. Int. J. Immunopharmacol. 14, 1–9.

Di Francesco, P., Falchetti, R., Gaziano, R., Lanzilli, G., Belogi, L., Ravagnan, G., Garaci, E., 1994. Differential effects of short-term or prolonged cocaine exposure on peripheral blood cells in mice. Life Sci. 54, 2015–2020.

Di Francesco, P., Falchetti, R., Gaziano, R., Lanzilli, G., Casalinuovo, I.A., Ravagnan, G., Garaci, E., 1999. Effects of cocaine administration to influenza virus-immunized mice on cytokine profiles of individual splenic CD4+ and CD8+ T cells. Clin. Exp. Immunol. 118, 428–434.

Gajewski, T.F., Joyce, T., Fitch, F.W., 1989. Antiproliferative effect of IFN-gamma in immune regulation: III. Differential selection of TH1 and TH2 murine helper T lymphocyte clones using recombinant IL-2 and recombinant IFN-gamma. J. Immunol. 143, 15–22.

Gan, X., Zhang, L., Newton, T., Chang, S.L., Ling, W., Kermani, V., Berger, O., Graves, M.C., Fiala, M., 1998. Cocaine infusion increases interferon-gamma and decreases interleukin-10 in cocaine-dependent subjects. Clin. Immunol. Immunopathol. 89, 181–190.

Havas, H.F., Dellaria, M., Schiffman, G., Geller, E.B., Adler, M.W., 1987. Effect of cocaine on the immune response and host resistance in BALB/c mice. Int. Arch. Allergy Appl. Immunol. 83, 377–383.

Inglot, A.D., Leszek, J., Piasecki, E., Sypula, A., 1994. Interferon responses in schizophrenia and major depressive disorders. Biol. Psychiatry 35, 464–473.

Kalivas, P.W., Pierce, R.C., Cornish, J., Sorg, B.A., 1998. A role for sensitization in craving and relapse in cocaine addiction. J. Pharmacol. 12, 49–53.

Kubera, M., Basta-Kaim, A., Skowron-Cendrzak, A., Mazur-Kolecka, B., Roman, A., Borycz, J., 1995. Effect of repeated amitriptyline administration to mice on the T lymphocyte proliferative activity and natural killer cell cytotoxicity. Pol. J. Pharmacol. 47, 321–326.

Kubera, M., Symbirtsev, A., Basta-Kaim, A., Borycz, J., Roman, A., Papp, M., Claesson, M., 1996. Effect of chronic treatment with imipramine on interleukin 1 and interleukin 2 production by splenocytes obtained from rats subjected to a chronic mild stress model of depression. Pol. J. Pharmacol. 48, 503–506.

Kubera, M., Holan, V., Basta-Kaim, A., Roman, A., Borycz, J., Shani, J., 1998. Effect of desipramine on immunological parameters in mice, and their reversal by stress. Int. J. Immunopharmacol. 20, 429–438.

Kubera, M., Maes, M., Holan, V., Basta-Kaim, A., Roman, A., Shani, J., 2001. Prolonged desipramine treatment increases the production of interleukin-10, an anti-inflammatory cytokine, in C57BL/6 mice subjected to the chronic mild stress model of depression. J. Affect. Disord. 63, 171–178.

Kubera, M., Filip, M., Basta-Kaim, A., Nowak, E., Budziszewska, B., Tetich, M., Holan, V., Korzeniak, B., Przegalinski, E., 2002. The effect of amphetamine sensitization on mouse immunoreactivity. J. Physiol. Pharmacol. 53, 233–242.

Le Gros, G., Ben-Sasson, S.Z., Sender, R., Finkelman, F.D., Paul, W.E., 1990. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing ells. J. Exp. Med. 172, 921–929.

Liu, Y., Peterson, D.A., Kimura, H., Schubert, D., 1997. Mechanism of cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. J. Neurochem. 69, 581–593.

Maes, M., Bocchio Chiavetto, L., Bignotti, S., Battisa Tura, G., Pioli, R., Boin, F., Kenis, G., Bosmans, E., de Jongh, R., Lin, A., Racagni, G., Altamura, C.A., 2000. Effects of atypical antipsychotics on the inflammatory response system in schizophrenic patients resistant to treatment with typical neuroleptics. Eur. Neuropsychopharmacol. 10, 119–124.

Ou, D.W., Shen, M.-L., Luo, Y.-D., 1989. Effects of cocaine on the immune system of Balb/c mice 52. Clin. Immunol. Immunopathol. 52, 305–312.

Pellegrino, T., Bayer, B.M., 1998. In vivo effects of cocaine on immune cell function. J. Neuroimmunol. 83, 139–147.

Pellegrino, T.C., Dunn, K.L., Bayer, B.M., 2001. Mechanisms of cocaine-induced decrease in immune cell function. Int. J. Immunopharmacol. 1, 665–675.

Pierce, R.C., Kalivas, P.W., 1997. A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res. Rev. 25, 192–216.

Przegalinski, E., Filip, M., Siwanowicz, J., Nowak, E., 2000. Effect of adrenalectomy and corticosterone on cocaine-induced sensitization in rats. J. Physiol. Pharmacol. 51, 193–204.

Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Rev. 18, 247–291.

Ruiz, P., Cleary, T., Nassiri, M., Steele, B., 1994. Human T-lymphocyte subpopulations and NK cell alterations in persons exposed to cocaine. Clin. Immunol. Immunopathol. 70, 245–250.

Scott, P., Kaufmann, S.H.E., 1991. The role of T-cell subsets and cytokines in the regulation of infection. Immunol. Today 12, 346–348.

Shen, M.L., Luo, Y.D., Hagen, K., Wu, Y.B., Ou, D., 1994. Immunomodulating activities of cocaine—evaluation of lymphocyte transformation related to other immune functions. Int. J. Immunopharmacol. 16, 311–319.

Stanulis, E.D., Jordan, S.D., Rosecrans, J.A., Holsapple, M.P., 1997. Disruption of Th1/Th2 cytokine balance by cocaine is mediated by corticosterone. Immunopharmacology 37, 25–33.

Van Dyke, C., Stesin, A., Jones, R., Chonthrapal, A., Seaman, W., 1986. Cocaine increases natural killer cell activity. J. Clin. Invest. 77, 1387–1390.

Wang, Y., Dennis, S.H., Watson, R.R., 1994. In vivo and in vitro cocaine modulation on production of cytokines in C57BL/6 mice. Life Sci. 54, 401–411.

Watson, E.S., Murphy, J.S., ElSolhy, H.N., ElSolhy, M.A., Turner, C.E., 1983. Effects of the administration of coca alkaloids on the primary immune responses of mice: interaction with tetrahydrocannabinol and ethanol. Toxicol. Appl. Pharmacol. 71, 1–13.

Weinberg, S.B., Schulteis, G., Fernando, A.G., Bakhit, C., Santos, G., 1988. Decreased locomotor activity produced by repeated, but not single, administration of murine-recombinant interferon-gamma in mice. Life Sci. 42, 1085–1090.

Weiss, S.H., 1989. Links between cocaine and retroviral infection. JAMA 261, 607–660.