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Immunosuppression Induced by a Conditioned Stimulus Associated With Cocaine Self-Administration

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Abstract. Cocaine addiction is known to impair immune system function, but the effects of repeated treatment with cocaine in a self-administration model, its withdrawal as well as reinstatement of cocaine-seeking behavior on cell-mediated immunity are not well known. Cocaine self-administered for 18 days induced a significant increase in spleen weight, plasma corticosterone levels, interleukin (IL)-10, and tumor necrosis factor-α production, while concanavalin A–stimulated proliferation responses of peripheral blood T-lymphocytes and interferon-γ production by splenic lymphocytes were not altered. After 10 days withdrawal from cocaine, reinstatement of cocaine seeking behavior induced either by a priming dose of the drug (unconditioned stimulus), by cue previously associated with cocaine self-administration (conditioned stimuli), or by both these stimuli evoked similar changes in several immunological parameters, for example, a decrease in relative spleen weight, proliferative activity of splenocytes, and their ability to produce IL-10. The results showed that the cue previously associated with cocaine suppressed some parameters of cell-mediated immunity to the same degree as re-exposure to cocaine. The present study provides the first evidence that alterations of immune status can be conditioned by environmental stimuli paired with cocaine administration.

Keywords: cocaine, extinction, relapse, self-administration, immune system

Introduction

Cocaine is one of the most powerful addictive substances in humans and its abusers are at a high risk of relapse. The factors responsible for cocaine relapse are not completely understood, but research on humans provides evidence that relapse to cocaine use or cocaine craving can be initiated by multiple triggers including self-administered drug or drug-associated environmental cues (1 – 3). In preclinical studies, relapse can be modeled in a reinstatement procedure in which laboratory animals are trained to self-administer drugs and then subjected to extinction training during which (in an operant version of this procedure) lever presses are not reinforced with drugs. Reinstatement of extinguished lever responding (the operational measure of drug seeking) is determined after such manipulations as noncontingent priming injections of the drug (4), exposure to cues associated with drug intake (5), or exposure to stress (6). The reinstatement model has good validity for modeling the activation of craving and arousal by environmental conditioned stimuli in drug-dependent individuals.

Recent studies point to a relationship between cocaine and increased susceptibility to infections (7, 8) and, on the other hand, between alterations in the immune function and conditioned environmental stimuli (9, 10). First, cocaine users have a higher incidence of HIV seroprevalence and hepatitis C and Herpes simplex virus-2 infection than other intravenous drug users. This increased susceptibility to viral infections can be explained by decreases in cell-mediated immune func-

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tion in cocaine addicts. Thus, cocaine suppresses the immune system, altering the function of natural killer (NK) cells, T cells, neutrophils and macrophages, and alters the ability of these cells to secrete immunoregulatory cytokines, for example, perturbing the balance of Th1 pro-inflammatory versus Th2 anti-inflammatory cytokines (11). These wide-ranging effects on the immune and neuroendocrine systems resemble an inflammatory "stress" response with upregulation of pro-inflammatory cytokines and stimulation of the HPA axis (12). Recent evidence indicates also that withdrawal from cocaine shares similarities with the stress response. On the other hand, using different conditioning situations, it was shown that re-exposure to a conditioned stimulus previously associated with different immunosuppressive stimuli reduced the severity of autoimmune diseases in animal models and in humans and prolonged survival of skin allograft (9, 13, 14).

The aim of this study was to examine whether repeated treatment with cocaine in a self-administration model, cocaine withdrawal, and reinstatement of cocaine-seeking behavior affects cell-mediated immunity. Cocaine seeking behavior was induced either by the priming dose of the drug ("unconditioned stimulus"), by a cue previously associated with cocaine self-administration ("conditioned stimulus"), or by both these stimuli. A "yoked" procedure was used in which rats were tested simultaneously in groups of two, with only one rat actively self-administering cocaine and the second receiving yoked injections of saline. The latter procedure allows us to access direct pharmacological effects of cocaine (cocaine *vs* saline groups). Many parameters of the immune system were examined: relative weight of immune organs, proliferative activity of splenocytes, and their ability to produce cytokines in response to mitogen stimulation.

Materials and Methods

Animals

Male Wistar rats (Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland) weighing 250 – 280 g at the beginning of the experiment were used. The rats were housed individually in a colony room maintained at 21 ± 2°C and at 40% – 50% humidity under a 12-h light-dark cycle (lights on at 06:00 h) and had free access to tap water and rodent chow. After a week quarantine, animals were deprived of water for 18 h and then trained to press a lever for water reinforcement on a fixed ratio (FR) 1 schedule of reinforcement in 2-h daily sessions. On the third day of the training, the number of responses required to produce water reinforcement was increased to a final value of five (a 5-response FR schedule of reinforcement). During this phase of training, the amount of water each animal received was restricted to that given during daily training sessions and after sessions for 10 min. All experiments were conducted during the light phase of the light–dark cycle (between 07:00 – 15:00 h) and were carried out in accordance with the *National Institutes of Health Guidelines for the Care and Use of Laboratory Animals* and were approved by the Bioethics Commission as compliant with Polish Law (21 August 1997).

Behavioral experiments

Drugs: Cocaine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile 0.9% NaCl and given either i.v. (0.05 ml/injection) or i.p. (1 ml/kg).

Self-administration procedures: The experimental design is shown in Table 1. The separate groups of rats were trained to self-administer cocaine ("self administration" group) and to reinstate cocaine seeking by either cocaine alone, cocaine + cue, or cue alone following cocaine self-administration associated with the conditioned stimulus ("cocaine seeking with cue") or

Table 1. Experimental design for self-administration procedures

Group	Group number	Experimental design		
		Maintenance	Extinction	Reinstatement
Self-administration	1	Yoked Saline + CS		
	2	Active Cocaine + CS		
Cocaine seeking (with cue)	3	Yoked Saline + CS	Saline	Saline + CS
	4	Active Cocaine + CS	Saline	Saline + CS
	5	Active Cocaine + CS	Saline	Cocaine + CS
Cocaine seeking (without cue)	6	Yoked Saline	Saline	Saline
	7	Active Cocaine	Saline	Saline
	8	Active Cocaine	Saline	Cocaine

CS: conditioned stimulus.

by cocaine alone following cocaine self-administration ("cocaine seeking without cue"). A "yoked" procedure was used in which rats were tested simultaneously in groups of two, with only one rat actively self-administering cocaine and the second receiving yoked injections of saline.

Apparatus: The procedures were conducted in commercially available, two-lever operant chambers (Med-Associates, St. Albans, VT, USA). Cocaine self-administration experiments were conducted in sixteen standard operant chambers (Med-Associates). Each chamber was equipped with a 24-V house light, located on the ceiling; two retractable levers on one wall; a water-filled dispenser mounted equidistantly between the levers; a white circular stimulus lamp illuminated by a 24-V bulb above each lever; and a tone generator. Lever pressing on one of the levers (defined as "active") resulted in drug delivery to the animal when the schedule (FR 5) requirements were met, whereas pressing on the other lever (defined as "inactive") were recorded but not reinforced. Completion of each FR 5 produced intravenous infusions of cocaine through liquid swivel (Instech, Plymouth Meeting, PA, USA) via an infusion pump (Model 3.33 RPM, Med-Associates). The position of the "active" and "inactive" levers remained unchanged throughout the study. A house light was on during the experimental sessions. The operant chambers were enclosed in ventilated, sound-attenuating cubicles (Med-Associates) and controlled by an IBM compatible computer using the MED Associates MED-PC software package.

Maintenance: Two days following initial lever-press training (described above) and free access to water, the rats were chronically implanted with a silastic catheter in the external right jugular vein, as described previously (15). Catheters were flushed every day with 0.1 ml of saline solution containing heparin (70 U/ml; Biochemie GmbH, Kundl, Austria) and 0.1 ml of cephazolin solution (10 mg/ml, Biochemie). Catheter patency was tested periodically with the ultrashort-acting barbiturate anesthetic methohexital (10 mg/kg, i.v.; loss of consciousness within 5 s). After a 10-day recovery period, all animals were deprived of water for 18 h and trained to press the lever to a fixed ratio 5 schedule of water reinforcement over a 2-h session. Then, the subjects were given access to cocaine during 2-h daily sessions performed 6 days/week (maintenance) and from that time they were given water *ad libitum*. The house light was on throughout each session. Each completion of five presses on the "active" lever complex (fixed ratio 5 schedule) resulted either in a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml) or a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml) and 5-s presentation of a stimulus complex (activation of the white stimulus light directly above the "active" lever and the tone generator, 2000 Hz; 15 dB above ambient noise levels). Following each injection, there was a 20-s time-out period during which responding was recorded but had no programmed consequences. Response on the "inactive" lever never resulted in cocaine delivery. Acquisition of the operant response lasted a minimum of 10 days until subjects met the following criteria: minimum requirement of 25 reinforcements with an average of 6 days and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of 10% of the average; this criterion was selected based on our prior experiments (15). Once stable rates of responding were established, rats (n = 5 rats/group) were either sacrificed to establish the effect of repeated treatment with cocaine on immunity ("self administration" group) or were subjected to extinction/reinstatement sessions.

Extinction and reinstatement: During extinction sessions, subjects had 2-h daily training sessions with no delivery of cocaine or the presentation of the conditioned stimulus. Once they reached the extinction criteria (a minimum of 10 extinction days with the responding on the active lever below 10% of the level observed during at least 3 consecutive maintenance days), the separate groups of rats (n = 5 rats/group, "cocaine seeking with cue" groups) previously self-administering cocaine paired with the conditioned stimulus were tested in 2-h sessions for response reinstatement induced by a noncontingent presentation of the self-administered reinforcer (10 mg/kg cocaine, i.p.) given immediately before testing together with a discrete contextual cue (tone + light previously paired with cocaine self-administration) presented contigently upon the animal's lever press or by a discrete contextual cue alone presented contingently upon the animal's lever press. The other groups of animals (n = 5 rats/group, "cocaine seeking without cue") previously self-administering cocaine without the conditioned stimulus were tested in 2-h sessions for response reinstatement induced by a noncontingent presentation of the self-administered reinforcer (10 mg/kg cocaine, i.p.) given immediately before testing. The reinstatement test was conducted only once.

Immunological studies

Preparation of cell suspensions: The rats were decapitated immediately after the last training session (5 animals from groups 1 and 2) or immediately after the 2-h session of reinstatement (groups 3 – 8, five animals from each group). Their spleens and thymuses were aseptically dissected and weighed. The spleens were gently crushed in a glass homogenizer. The spleen cells

were resuspended in the RPMI-1640 medium (Sigma-Aldrich, Steinheim, Germany), and were centrifuged at 500 × *g* for 5 min. The cell pellets were resuspended in the same medium supplemented with antibiotics (50 μg/ml of penicillin, 50 μg/ml of streptomycin), a 10% fetal bovine serum and 2 mM L-glutamine (all reagents were obtained from Sigma).

Proliferative response of splenocytes to mitogen stimulation in vitro: The proliferative response of spleen cells was described earlier by Kubera et al. (16). 4×10^6 splenocytes per ml were stimulated with 0.6 or 2.5 μg/ml of concanavalin A (Con A; Sigma Chemical Co., St. Louis, MO, USA). The cells 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 10 μl (0.5 μCi) of [^{3}H]-thymidine per well (sp. act. = 6.7 Ci/mmol; MP, Biomedicals, Inc., Irwine, CA, USA) 16 h before the end of incubation. The cultures were harvested with an automatic cell harvester (Scatron, Lier, Norway), and [^{3}H]-thymidine incorporation was estimated with a liquid scintillation counter (LS 6500; Beckman, Ramsey, MI, USA).

Determination of cytokines: Rat splenocytes were tested for their ability to produce interleukin (IL)-1, IL-10, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ after mitogen stimulation. Splenocyte suspensions were seeded at a concentration of 4×10^6 cells/ml in 24-well Corning tissue culture plates and were then stimulated with a Con A solution (2.5 μg/ml). Cell-free supernatants were collected 72-h later and stored at –20°C. All the enzyme-linked immunosorbent assays (ELISA) were based on monoclonal–monoclonal antibody pairs and were performed using DuoSet ELISA Development Kits (R&D Systems, Inc., Minneapolis, MN, USA)

The intra-assay CV values for both those analyses did not exceed 10%. The viability of cells was checked with trypan blue.

Corticosterone: The blood was collected on EDTA, centrifuged at 800 × *g* for 15 min, and the supernatant was removed and stored at –20°C until analysis. The CORT level was measured by radioimmunoassay after ethanol precipitation of plasma proteins, as described previously (17). 1,2,6,7-[^{3}H]-Corticosterone (sp. act. = 85 Ci/mmol) was purchased from the Radiochemical Centre Amersham (Amersham, UK) and the antiserum for corticosterone was obtained from the UCB-Bioproducts SA (Braine-l'-Alleud, Bergium). CORT content was calculated using a log-logit transformation. The assay sensitivity was 10 pg/tube. Intra- and inter-assay coefficients of the variation were lower than 5% and 8%, respectively.

Statistical analyses

The data are each shown as a mean ± S.E.M (behavioral studies) or a mean ± S.D. (immunological and corticosterone studies). In cocaine self-administration procedures, the number of the active lever presses (including time-out responding) for each group was analyzed by separate one-way analyses of variance (ANOVAs). Post hoc Dunnett's test was used to analyze differences between group means. Immunological and corticosterone data were evaluated by a one-way ANOVA followed by individual comparison using the Tukey honest significant differences test. The criterion for statistically significant differences was set at $P<0.05$.

Results

Behavioral studies

Rats showed stable active lever responding (135 ± 17; Fig. 1, upper panel) during the last 6 self-administration maintenance sessions with an acquisition criterion requiring that the rate of active lever presses varied by less than 10%. The animals had self-administered ca. 25 injections of cocaine with the daily mean cocaine intake of about 12.5 mg/kg (data not shown).

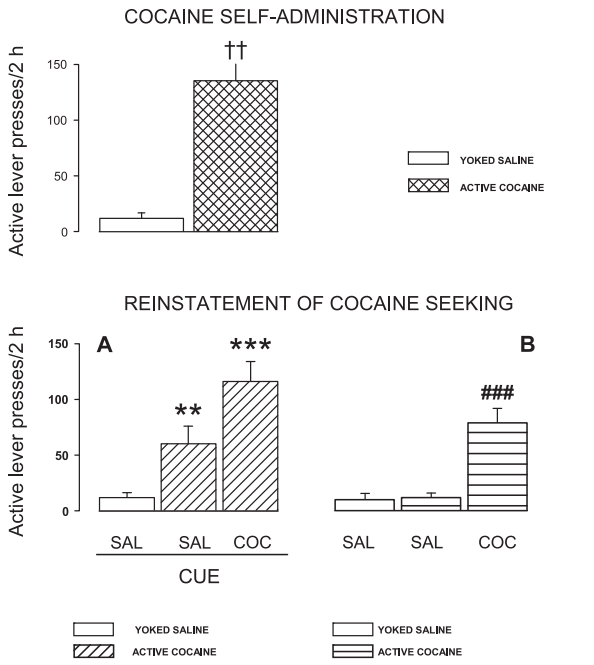

Fig. 1. Active lever presses during the maintenance (upper panel) and the reinstatement of cocaine seeking (bottom panels) induced by either drug-associated cue (A) or cocaine (COC: B) in rats self-administering cocaine and yoked saline (SAL) controls. During maintenance, active lever responses resulted in delivery of a cocaine infusion (0.5 mg/kg per infusion) and simultaneous presentation of a light + tone stimulus complex. Data are each expressed as a mean ± S.E.M. $^{\dagger\dagger}P<0.01$ *vs* yoked saline; $^{**}P<0.01$, $^{***}P<0.001$ *vs* SAL + cue; $^{\#\#\#}P<0.001$ *vs* SAL.

After 10 days of extinction, the presentation of self-administered reinforcer (10 mg/kg cocaine, i.p.) together with a discrete contextual cue or a discrete contextual cue alone induced a significant increase in active lever presses ($F(2,12) = 9.12$, $P<0.01$) in rats previously self-administering cocaine paired with the conditioned stimulus ("cocaine seeking with cue" group) as seen in Fig. 1 (lower left panel, A).

After 10 days of extinction, the presentation of self-administered reinforcer (10 mg/kg cocaine, i.p.), but not saline injection, induced a significant increase in active lever presses ($F(2,12) = 6.44$, $P<0.01$) in rats previously self-administered cocaine without the conditioned stimulus ("cocaine seeking without cue" group) as seen in Fig. 1 (lower right panel, B).

Immunological studies

Thymus and spleen weight: There were no significant differences in body weight between all studied groups. There were significant differences in the relative spleen and thymus weight (the weight of the spleen or thymus divided by the body weight) after 18 days of exposure to CS and cocaine (respectively $F = 41.9$, $df = 1/8$, $P<0.0001$ and $F = 65$, $df = 1/8$, $P<0.002$). Figure 2 shows a the significant increase, by ca. 89%, in the relative spleen weight and significant decrease, by ca. 25%, in the relative thymus weight in group 1 in comparison to group 2, whereas the main body weight of animals in groups 1 and 2 were, respectively, 365 ± 18 and 356 ± 29 g.

There were significant differences in the relative spleen weight after reinstatement of "cocaine seeking with cue" as well as after "cocaine seeking without cue". A priming dose of the drug (unconditioned stimulus) in group 8 increased relative spleen weight by about 104% in comparison to saline-treated control group 6, whereas the main body weight of animals in groups 6 and 8 were, respectively, 378 ± 34 and 363 ± 8 g (Fig. 3).

Cue previously associated with cocaine self-administration (group 4) as well as cocaine administration simultaneously with conditioning stimuli (group 5) increased relative spleen weight by about 56% and 60% in comparison to saline-treated control group 3 as it was shown on Fig. 3 (main body weight in group 3, 4, and 5 were respectively 365 ± 30, 361 ± 32, and 350 ± 25 g).

Proliferative activity of splenocytes: It has been shown that 18-day exposure to cocaine did not affect proliferative activity of splenocytes for both concentrations of Con A (group 1 *vs* group 2, data not shown).

Withdrawal from self-administered cocaine (group 7) did not affect proliferative activity of splenocytes in

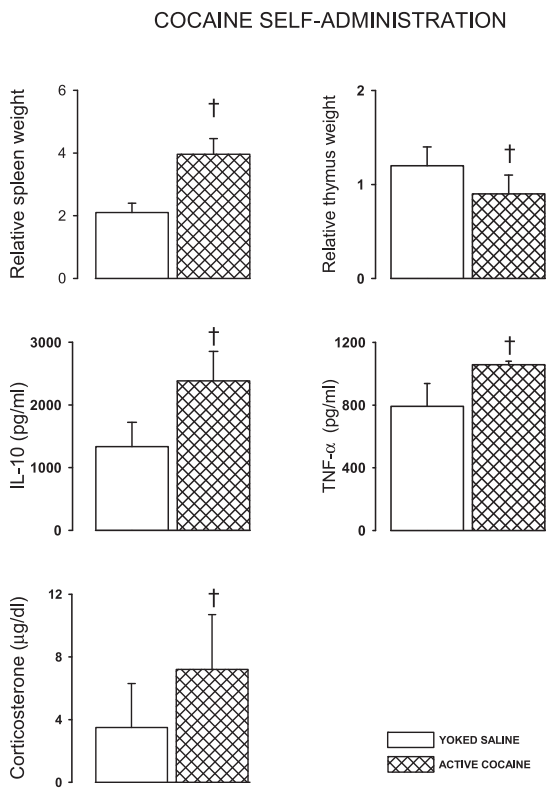

Fig. 2. Effect of self-administered cocaine (0.5 mg/kg per infusion) on the relative spleen and thymus weight, IL-10 and TNF-α productions, and plasma corticosterone level. The rats were decapitated immediately after the last training session (n = 5 animals/group). The relative spleen and thymus weight was estimated as weight of the spleen or thymus in mg divided by body weight in g. The ability of splenocytes stimulated by Con A to produce cytokines was determined in duplicate for each animal. Data are each expressed as a mean ± S.D. $^{\dagger}P<0.05$ *vs* yoked saline.

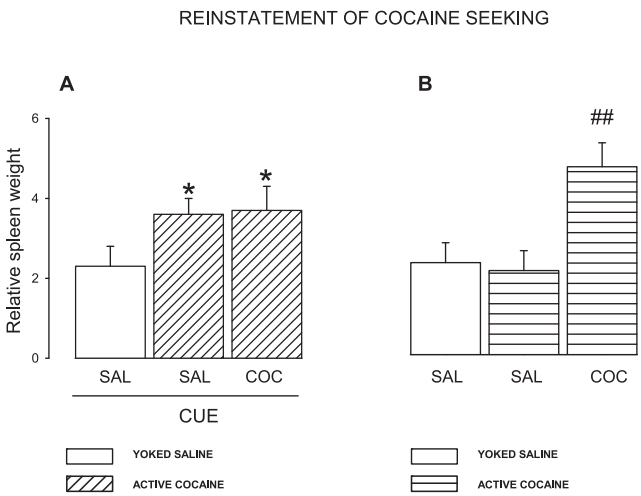

Fig. 3. Effect of reinstatement of cocaine seeking following 10-day withdrawal in rats self-administering cocaine (0.5 mg/kg per infusion) on relative spleen weight. Reinstatement was evoked by either a cue, a cue combined with cocaine (10 mg/kg, i.p.) (A), or by cocaine (10 mg/kg, i.p.) (B). Data are each expressed as a mean ± S.D. $^{*}P<0.05$ *vs* saline (SAL) + cue; $^{\#\#}P<0.01$ *vs* SAL.

comparison to exposure of the animals to saline (group 6), whereas reinstatement of cocaine seeking by conditioned stimulus (group 4) as well as cocaine administration simultaneously with conditioning stimuli (group 5) or without conditioning stimuli (8) decreased proliferative activity of splenocytes in comparison to the appropriate controls (Fig. 4).

Lymphokine production by splenic lymphocytes: The production of IL-10 and TNF-α was significantly increased after 18 days of exposure to cocaine (Fig. 2), whereas production of IL-1 and IFN-γ was not changed (data not shown).

Ten-day withdrawal from self-administered cocaine (group 7) did not affect IL-10 production, whereas reinstatement of cocaine seeking by conditioned stimuli alone (group 4) as well as exposure to cocaine simultaneously with conditioning stimuli (group 5) or without conditioning stimuli (8) decreased production of IL-10 by splenocytes in comparison to the appropriate controls (Fig. 5).

Ten-day withdrawal from self-administered cocaine (group 7) decreased IFN-γ production in comparison to the yoked-saline–treated control group (group 6). Reinstatement of cocaine seeking by exposure to conditioned stimuli without cocaine administration (group 4) also significantly decreased IFN-γ production in comparison to the control group (group 3). On the other hand, reinstatement of cocaine seeking by exposure to cocaine simultaneously without the cue (group 5) or to cocaine without the cue (group 8) did not change IFN-γ production in comparison to the

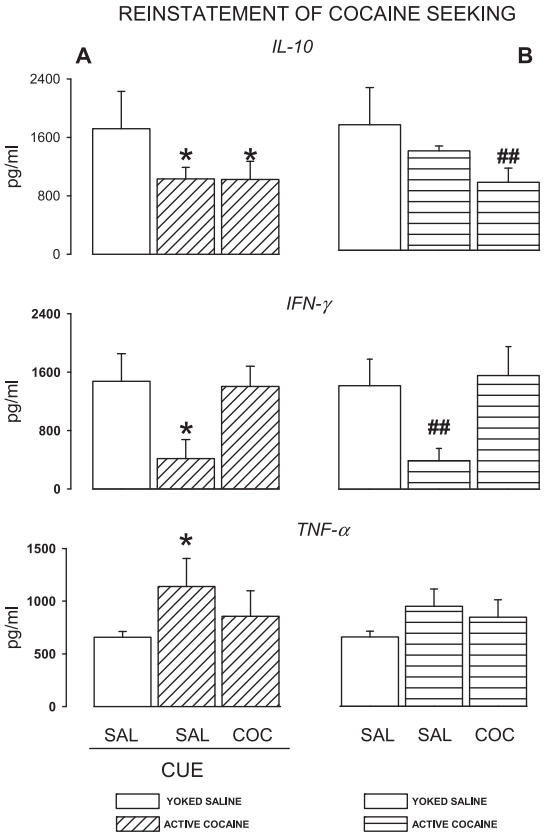

Fig. 5. Effect of reinstatement of cocaine seeking following 10-day withdrawal in rats self-administering cocaine (0.5 mg/kg per infusion) on IL-10, IFN-γ, and TNF-α production. Reinstatement was evoked by either a cue, a cue combined with cocaine (10 mg/kg, i.p.) (A), or by cocaine (10 mg/kg, i.p.) (B). Data are each expressed as the mean ± S.D. *P<0.05 *vs* saline (SAL) + cue; $^{\#\#}P$<0.01 *vs* SAL.

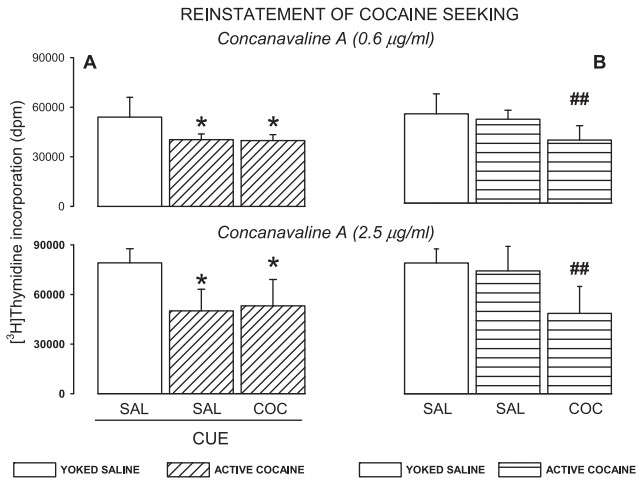

Fig. 4. Effect of reinstatement of cocaine seeking following 10-day withdrawal in rats self-administering cocaine (0.5 mg/kg per infusion) on proliferative activity of splenocytes stimulated by concanavalin A. Reinstatement was evoked by either a cue, or a cue combined with cocaine (10 mg/kg, i.p.) (A), or by cocaine (10 mg/kg, i.p.) (B). Data are each expressed as a mean ± S.D. *P<0.05 *vs* saline (SAL) + cue; $^{\#\#}P$<0.01 *vs* SAL.

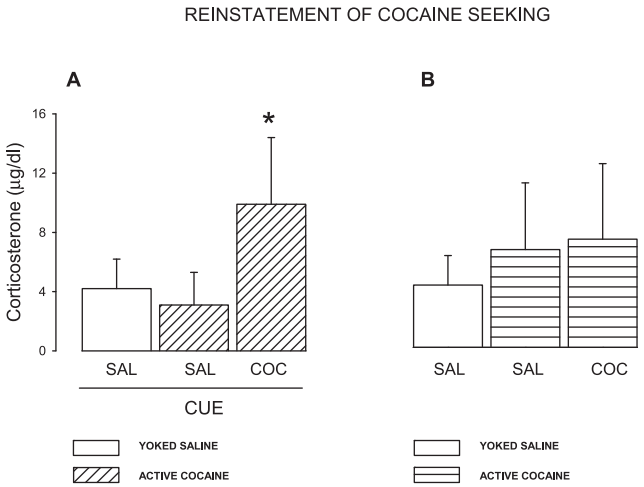

Fig. 6. Effect of reinstatement of cocaine seeking following 10-day withdrawal in rats self-administering cocaine (0.5 mg/kg per infusion) on plasma corticosterone level. Reinstatement was evoked by either a cue, a cue combined with cocaine (10 mg/kg, ip.) (A), or by cocaine (10 mg/kg, i.p.) (B). Data are each expressed as a mean ± S.D. *P<0.05 *vs* saline (SAL) + cue.

appropriate controls (Fig. 5).

TNF-α production was increased in all experimental groups, although a statistically significant effect was observed only after reinstatement of seeking behavior after application of conditioned stimuli alone without exposure to cocaine (group 4) (Fig. 5).

Plasma corticosterone concentration: The plasma corticosterone concentration was significantly increased after eighteen days of exposure to cocaine (group 2 *vs* group 1).

Reinstatement of cocaine seeking by exposure to cocaine without (group 8) or with cue (group 5) increased corticosterone concentration, although only the latter result is statistically significant (Fig. 6).

Discussion

The main finding of the present study is demonstration that the immune alterations can occur following exposure of animals to the conditioned stimulus previously paired with cocaine self-administration. Exposure of animals to conditioned stimuli (group 4) after 18 trials when conditioned stimuli had been paired with cocaine self-administration and 10 days of withdrawal from cocaine induced similar changes in cell-mediated immunity as cocaine administration with cue (group 5) or without cue (group 8) after 10 days of withdrawal from cocaine. In groups 4, 5, and 8, the mitogenic responsiveness and IL-10 production by splenocytes stimulated by Con A were decreased, whereas spleen weight was increased. Moreover production of TNF-α was enhanced in groups 5, 8, and 4, but these effects reached statistical significance only in group 4.

The main "conditioned" change in immunity seems to be decrease of proliferative activity of splenocytes. Reduction in the proliferative activity of splenocytes was described in several papers after single but not repeated exposure to cocaine (18 – 21), so splenocytes of mice exposed to cue and/or cocaine after 10 days withdrawal from cocaine respond like splenocytes of mice after acute cocaine treatment.

Cocaine shows multiple pharmacological and physiological effects that could affect immune cell function. Cocaine binds to monoamine reuptake pumps present on neurons and immunocytes, preventing removal of serotonin, dopamine, and norepinephrine from the synaptioc cleft and microenviroment of lymphoid cells. Cocaine acts also as a muscarinic cholinergic antagonist and activates the HPA axis (21).

To the best of our knowledge, cocaine was shown here for the first time to evoke conditioning of the immune response. Others investigators showed that conditioning of cell-mediated immunity was affected by brain excitotoxic lesions, and the insular cortex was essential for acquiring and evoking the conditioned response. Thus, central catecholamines seem to be essential, and glutamate, but not GABA, is also required at the recall stage, whereas the central cholinergic and serotonergic system are required at the association and recall stages (22). These authors tried also to identify a discrete neural network that modulates peripheral immune functions and which can possibly be elicited by psychosocial context. It is currently believed that the major neuronal efferent pathways through which memories could affect peripheral immune functions are the neocortical – sympathetic – immune axis, including limbic and hypothalamic relays; hypothalamus – pituitary – adrenal immune axis; and the brainstem–vagus – cholinergic pathway.

In conditioned immunosuppression of the splenocyte proliferative activity, a particular role may be played by adrenergic stimulation via the splenic nerve and β-adrenergic receptors present on splenocytes. To this end it was shown that that chemical sympathectomy via 6-OHDA or β-adrenergic antagonist propranolol attenuated the conditioned suppression of splenocytes proliferation induced by association of conditioned stimulus with cyclosporine A, morphine, or electric footshock (23 – 25). Recently it was reported that conditioned modulation of neutrophil activity is also catecholamine-dependent (26).

It should be mentioned that the metabolism of dopamine and serotonin in various brain areas and the metabolism of noradrenaline in the brain stem of cocaine withdrawn animals were assessed in our former study. The results showed that stimuli associated with cocaine availability activated the catecholaminergic system in these animals (27). Therefore, one can speculate that in the present study, cocaine induced conditioned immunosuppression in spleen is also catecholamine-dependent. Moreover, cocaine activates the CRH system, leading to increased ACTH secretion, causing overstimulation of the adrenal cortex and an elevation of cortisol in humans and corticosterone in rats (28). On the other hand, monoamines, CRH, ACTH, and corticosterone exert multiple effects on the immune system, and the effect of these modulators depends on both their concentration in the lymphocyte microenvironment as well as the state of activation of target cells. In vitro administration of glucocorticoids and catecholamines in doses typical for stress decrease T- and B-cell proliferation (29), whereas our previous study showed that such doses of cortisol (10^{-6} M and higher) strongly decreased IL-10 production (30). Thus, it is not unlikely that cocaine–self-administration for reinstatement of cocaine seeking behavior in groups 5 and 8 or signal associated with

cocaine availability (group 4) increases glucocorticoid and/or catecholamine levels in the splenocyte environment to the concentration able to inhibit IL-10 production, although in the present study, the increase in corticosterone level was observed only in group 5.

The present study showed that besides proliferative activity of splenocytes, also spleen weight could be affected by the signal associated with cocaine availability. The increase in the spleen weight might result from the enhanced blood flow due to cocaine-induced vasodilatation.

In the present experiments, chronic cocaine administration to rats increased IL-10 and TNF-α production and had no effect on IL-1 and IFN-γ production (group 2 *vs* group 1, Fig. 2). Also Wang et al. (31) observed an increase in TNF-α production after six weeks of cocaine administration to C57BL/6 mice, whereas Gardner et al. (32) reported an increase in IL-10 production after prolonged cocaine administration connected with σ-receptor activation. Recent evidence indicates that glucocorticoids and catecholamines, at low but not "typical for stress" or pharmacological doses may up-regulate systemic IL-10 and local TNF-α production (33).

Both immunosuppression induced by simple cocaine administration as well as stimulation of some factors by chronic cocaine may result from changes in the neurohormonal environment that induces time-dependent alterations in the lymphocyte subpopulations and/or expression of some receptors present on these cells.

The significant decrease in thymus weight observed by us in rats treated for 18 days with cocaine may result from the elevated glucocorticoids levels, which in turn leads to lysis of glucocorticoid–sensitive immature thymocytes. Similar decreases in relative thymus weight were observed by us after three weeks of application of chronic mild stressors to C57BL/6 mice (34) and in sensitizated to cocaine Swiss mice (18). Moreover it was shown that, unlike in case of other stressors, tolerance does not develop to the neuroendocrine effects of repeated cocaine injections. This is evidenced by Avila et al. (35) who observed a significant increase in corticosterone levels after 7 days of cocaine administration and Sarnyai et al. (36) who showed increases in basal levels of corticosterone after 3 weeks of cocaine administration.

Ten-day withdrawal from cocaine did not affect proliferative activity of splenocytes and IL-10 production, but significantly inhibited IFN-γ production (group 7, Fig. 6). In the present study, we did not observe an increase in IFN-γ production by the signal of cocaine availability (group 4, Fig. 6). Such long-lasting inhibition of IFN-γ production may have negative influence on the activity of natural killer cells playing the main role in elimination of tumor cells in early stages of carcinogenesis.

Both clinical and preclinical findings indicate that the behavioral response to cocaine reinstatement of cocaine seeking behavior depend on the drug ability to block the dopamine transporter, thereby increasing the extracellular dopamine concentration within the mesocorticolimbic system and indirect activation of dopamine D_1 and D_2 receptors (for reviews, see refs. 37 – 39).

A large body of evidence indicates a major role of the activation of the dopaminergic and glutamatergic pathways in the nucleus accumbens, prefrontal cortex, ventral tegmental area, amygdala, and ventral hippocampus in the drug-priming and cue-induced reinstatement of cocaine seeking (for reviews, see refs. 40 – 44).

Further studies are required to elucidate potential physiological mechanisms that may be involved in the conditioned immunomodulatory effects and include the changes induced by cocaine on autonomic nervous system activity, neuroendocrine hormone secretion, and activations of dopaminergic and glutaminergic pathways in the central nervous system as well as on the changes in plasma monoamine concentrations.

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