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# Chronic Amphetamine Facilitates Immunosuppression in Response to a Novel Aversive Stimulus: Reversal by Haloperidol Pretreatment

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BASSO, A. M., G. GIOINO, V. A. MOLINA AND L. M. CANCELA. *Chronic amphetamine facilitates immunosuppression in response to a novel aversive stimulus: Reversal by haloperidol pretreatment.* PHARMACOL BIOCHEM BEHAV **62**(2) 307–314, 1999.—The effect of chronic *d*-amphetamine sulfate (AMPH) treatment (nine daily injections, 2 mg/kg IP) on subsequent foot shock stress-induced immunological response was investigated. In addition, the potential role of a dopaminergic (DA) mechanism in the development of chronic AMPH-induced changes in stress-influenced immune responses was characterized. Exposure to foot shock stress decreased the percentage of T-lymphocytes, and reduced the delayed-type hypersensitivity reaction (DTH) in chronically AMPH-pretreated rats relative to vehicle-treated controls. Both of those stress-induced immunosuppressive responses were no longer evident when AMPH-pretreated rats were injected with haloperidol (HAL, 1 mg/kg IP) 30 min prior to each daily AMPH injection. The present findings are indicative of a modulatory role for dopamine in the facilitating process induced by AMPH on stress-induced immunosuppressive effects. © 1999 Elsevier Science Inc.

Amphetamine Sensitization Stress Haloperidol Dopamine Immunosuppression Lymphocytes Delayed-type hypersensitivity reaction Hemagglutinin titers Rats

IT is well-known that repeated exposure to psychostimulants is characterized by enhanced responsiveness to the behavioral, electrophysiological, and neurochemical effects of subsequent administration of the same psychostimulant or a related drug (27,35,43). This phenomenon of enhanced behavioral effects after repeated psychostimulant administration is called sensitization or reverse tolerance, and has been shown to be long lasting (2,27,35,43). Sensitization to nonpharmacological stressors has also been observed, and the effects of chronic psychostimulants and stress have been shown to cross-sensitize, as demonstrated in numerous behavioral and neurochemical studies (2,22,24,27,37). Thus, it was reported that following repeated treatment with AMPH, the neurochemical and behavioral responses to stress that involve the activation of central DA pathways, are potentiated (27,44). The phenomenon of sensitization is an example of neuronal plasticity, and shares many of the characteristics of other forms of neuronal plasticity. Much evidence has been gathered regarding the neurobiological mechanisms underlying the expression of behavioral sensitization (27,31,32,54). It is hypothesized that a critical component after a chronic psychostimulant treatment is the capacity to enhance mesolimbic DA transmission, without a compensatory attenuation in DA activity. Along those lines, it has been shown that the augmented behavioral effects of psychomotor stimulants are generally associated with an enduring activation of the mesocorticolimbic DA system (27,43,54). One of the most widely observed changes that occurs during behavioral sensitization is an increase of dopamine release upon an acute challenge with a psychostimulant drug, or other stimulus (43). Although the behavioral sensitization process

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has been extensively studied, the exact processes by which repeated exposure to stress or AMPH persistently modifies the reactivity of DA neurons is presently unknown.

Several studies have attempted to elucidate the pharmacology of sensitization, using different DA receptor antagonists. Pretreatment with haloperidol (HAL), a nonselective DA antagonist, prevented the development of psychostimulant sensitization (28,31,33,38,53). More recent studies using selective $D_2$ DA antagonists have yielded contradictory results. However, most of the experiments have found that $D_2$ DA receptor antagonists failed to prevent psychostimulant-induced sensitization (51,54). However, there is a general agreement that $D_1$ selective DA antagonists, such as SCH-23390, prevent the development of sensitization to AMPH (15,51).

There is growing evidence demonstrating a regulatory role of the central nervous system (CNS) on the functioning of the immune system through various neuropeptides, neurohormones, and neurotransmitters (7,16,17). Numerous studies have revealed that stress can modify the susceptibility of animals to a wide variety of diseases, including neoplasia, by altering immune function. In general terms, exposure to an acute stress induces immunosuppression observed among others, as a decreased antibody production, inhibition of T-lymphocyte proliferation, decreased natural killer cell activity, facilitation of tumor appearance, and reduction of tumor rejection (29,34,39,47,48,52). Behavioral observations during or in response to a novel stressor, as well as studies performed to evaluate the immunological response to an aversive experience, have demonstrated that the effects of stress can be markedly different, depending on the chronic stress paradigms previously applied (4,5,8,9).

Interestingly, drug addicts are also known to be highly susceptible to bacterial, viral, and fungal infections, and to have important deficits in the immune function. Considerable evidence also now suggests that chronic administration of drugs of abuse compromises the immune function in both humans and laboratory animals, enhancing the vulnerability to several infectious diseases (6,10). Drugs such as psychostimulants and opiates have been demonstrated to affect the immune system, either directly or indirectly. For instance, acute and chronic morphine administration in experimental animals and in humans usually produces immunosuppression, demonstrated by reduction in phagocytosis, antibody production, blastogenic response to mitogens, cellular immunity, natural killer cell activity, and lymphokine production (50,55). Also, it has been shown that an acute dose of a psychostimulant drug such as AMPH suppresses lymphocyte mitogenic responses to mitogens, natural killer cell activity, and the production of cytokines in rodents (23,40,42).

The abuse of psychostimulant drugs such as AMPH in humans results in psychotic symptoms similar to paranoid schizophrenia (19,36). Furthermore, abnormalities in immunologic functioning have been identified in psychiatric disorders such as manic–depressive psychosis and schizophrenia (14,21,49). However, relatively little is known about the influence of chronic treatment with a psychostimulant drug on the immunological response related to subsequent exposure to an aversive stimulus in experimental animals. Therefore, it seems reasonable to hypothesize that chronic AMPH administration could influence the effect of stress on the functioning of the immune system. Because considerable evidence supports a critical role of central DA systems in the development of behavioral sensitization to both stress and psychostimulants, a dopaminergic mechanism could also be implicated in the effects of stress on the immune system following repeated AMPH administration. Therefore, the purpose of this study was to determine whether a prior chronic administration with AMPH could influence the immunological response to a subsequent aversive experience, and whether or not a dopaminergic mechanism is involved in the potential effect of chronic AMPH on the immune response to stress. Previous findings from this lab have shown that acute exposure to an aversive event (i.e., foot shock) resulted in a clear decrease in the percentage of T-lymphocytes and in the DTH reaction in unstressed rats (4,5), while no discernible effect was observed in the percentage of B-lymphocytes and in the hemagglutinin titer against sheep red blood cells (SRBC). However, in regard to the humoral immune response, controversial data exist in the literature (26,34). Therefore, the percentage of T- and B-lymphocytes, and cellular and humoral immune response to herologous antigens such as SRBC were the immunologic parameters evaluated in the present study.

## METHOD

### *Animals*

Adult male Wistar rats weighing 250–350 g were used in these experiments. Rats were maintained in groups of 6, at 22 ± 20°C under a 12 L:12 D cycle (lights on at 0700 h), with free access to food and water. All rats were acclimated to the experimental room for at least 1 week before the start of the experiments.

### *Drugs*

For all experiments d-amphetamine sulfate (AMPH) was dissolved in an isotonic saline solution (0.9% NaCl), which was also used for vehicle control injections. Haloperidol (HAL) (Droguería Prest, Yugoslavia) was dissolved in a 1% V/V acetic acid solution, and the pH was then adjusted to 5.5–5.7 with addition of a 0.1 N NaOH solution. All injections were administered intraperitoneally (IP) in a volume of 1 ml/kg.

### *Foot Shock Stress Apparatus and Protocol*

A chamber measuring 25 × 23 ×20 cm served as the shock apparatus. It had a grid floor of stainless steel rods through which scrambled electric shocks could be delivered via a shock generator. The stress protocol consisted of 30 min of foot shock exposure. The amplitude of the shock was 2 mA, and the shock duration was 3 s. Shocks were presented according to a variable interval schedule, with an average of one shock/min. This protocol was selected based on preliminary experiments that showed that this schedule of shock did not induce immunosuppression, and thus allowed the observation of the chronic AMPH-induced sensitization process on immune response after foot shock (Basso et al., unpublished observation).

### *Immunization*

Sheep red blood cells (SRBC) were washed three times in phosphate-buffered saline (PBS), pH = 7.2, and made up to an appropriate concentration for injection. Animals were immunized intraperitoneally (IP) with $1 \times 10^9$ cells in a volume of 1 ml on day 5 of the drug treatment. On day 8, were immunized intravenously (IV) through the tail vein with $1 \times 10^9$ cells in a volume of 0.2 ml. The concentration of SRBC was chosen based on preliminary studies from this laboratory,

which revealed that this dosage led to a submaximal antibody titers and a positive DTH (4,5).

*Immunologic Assays*

*Determination of the percentages of T- and B-lymphocytes.* Animals were bled by cardiac puncture under ether anesthesia and the blood was collected into heparinized syringes. Peripheral blood lymphocytes were isolated by a Ficoll-Hypaque density gradient centrifugation. The blood was diluted 1:1 with PBS, and 6 ml of the resulting solution was layered over 3 ml of Ficoll-Hypaque and centrifuged at 400 $\times$ *g* for 30 min at 18–20°C. Interface lymphocytes were removed and washed twice in PBS by centrifugation at 100 $\times$ *g* for 10 min. The third washing was conducted with PBS and 10% sodium azide ($NaN_3$) diluted 1:100.

The measurement of T-cells was based on surface markers known to associate with a different antibody; accordingly, OX19 monoclonal antibody directed against rat total T-lymphocytes (Bioproducts for Science Inc Lab.) was used. In brief, 1–2 $\times$ $10^6$ cells were incubated with 20 μl of a saturating concentration of monoclonal antibody (dilution 1:100) at 4°C for 30 min. After three washes with PBS and $NaN_3$, cells were incubated for 30 min at 4°C with fluorescein isothiocyanate-labeled polyvalent rabbit antimouse Ig G (Sigma Chemical Co., St. Louis, MO), washed three times, and the percentage of T-lymphocytes assayed with an immunofluorescence microscope.

For the measurement of B-cells, lymphocytes that were obtained as described above were incubated for 40 min at 4°C with fluorescein isothiocyanate-labeled polyvalent rabbit antirat Ig M and Ig G (Zymed Lab.), and washed three times. The percentage of B-cells was then determined with an immunofluorescence microscope.

To estimate the proportion of T- and B-cells, we counted 20 fields containing approximately 200 cells. The investigator who conducted the recount was blind with regard to the treatment condition of each animal.

*Cell-mediated immune response: Delayed-type hypersensitivity reaction (DTH).* Animals previously subjected to the method of immunization described above were evaluated in this assay. Delayed cutaneous hypersensitivity was elicited 8 days following the first immunization, a time point that corresponded to the fourth day after completion of the drug treatment. Rats were challenged with 1 $\times$ $10^8$ SRBC in 0.1 ml PBS under the right hind footpad; the left footpad received PBS alone. Reactions were assessed 24 h later, by measuring the increase in dorsoventral thickness of the test footpad over the control footpad, using a vernier caliper. Challenges and assessments of DTH reactions were conducted under ether anesthesia in Experiments 1 and 2. To discard a possible influence of ether on immune function, Experiment 2 was also performed under halothane anesthesia (3–4% in oxigen). After induction, animals were continuously maintained at a proper level of anesthesia, as defined by absence of the corneal reflex, by 1–2 % halothane. All measurements were conducted by the same investigator, and results were expressed as specific increases in footpad thickness, according to the following formula:

$$\text{DTH (mm)} = (\Delta\text{SRBC footpad} - \Delta\text{control footpad}) \times 10$$

*Humoral immune response: Determination of serum hemagglutinin titers.* Rats were first anesthetized with ether and blood was then collected by cardiac puncture and allowed to clot. Blood samples were centrifuged at 1000 rpm for 10 min. The serum supernatant was then collected, and complement was inactivated at 56°C for 30 min. Hemagglutinin titers to SRBC were estimated by serial dilutions of inactivated serum in PBS, and a 1% SRBC solution in microtiter plates. The highest dilution at which aggregation of SRBC was evident was considered to be the antibody titer, and was expressed as $\log_{10}$ of the reciprocal of the antibody titer.

*Experimental Procedure*

*Experiment 1: Effects of foot shock exposure on immunologic parameters in animals previously submitted to chronic AMPH- treatment.* Rats were chronically injected with AMPH (2 mg/kg/day IP) or saline (SAL) over 9 consecutive days. Four days after the last AMPH injection, all animals were placed in the foot shock chamber, and half of the animals of each group received foot shock. The remaining groups were placed in the apparatus without receiving shock. Immediately after shock exposure, all animals were bled by cardiac puncture under ether anesthesia. The blood was collected into heparinized syringes and the percentages of T- and B-lymphocytes were determined. In this study, the experimental groups consisted of: rats chronically injected with SAL without receiving the stressor, rats chronically injected with SAL and subsequently exposed to foot shock stress, rats chronically injected with AMPH and not exposed to the stressor and rats chronically injected with AMPH and subsequently exposed to the foot shock.

Another group of rats, which were divided into the same treatment groups, was immunized with SRBC on day 5 and again on day 8 of the 9-day AMPH treatment. To evaluate the DTH reaction, all rats were challenged with 1 $\times$ $10^8$ SRBC in 0.1 ml of PBS, injected intradermically under the foot pad, 96 h after the last AMPH or SAL injection. Two hours later, half of the animals assigned to each group received the shock, and the others remained in the chamber without receiving the shock. The increase in footpad thickness 1 day after the challenge with SRBC was evaluated with a vernier caliper. In addition, animals were bled by cardiac puncture without anticoagulant, and serum hemagglutinin titers against SRBC were estimated on heat-inactivated samples.

*Experiment 2: Effect of HAL on stress-induced immunosuppression induced by chronic AMPH-treatment.* Animals were pretreated daily with HAL 1 mg/kg, IP, or SAL, 30 min prior to each daily AMPH or SAL treatment throughout the entire chronic drug regimen. Accordingly, in the present experiment animals were randomly distributed into eight groups derived from a factorial design, where the factors under consideration were: HAL pretreatment (HAL or SAL), chronic drug treatment (AMPH or SAL), and shock delivery (shock or no shock). In summary, the experimental groups were as follows: SAL-SAL-no shock ; SAL-SAL-shock ; HAL-SAL-no shock ; HAL-SAL-shock ; SAL-AMPH-no shock; SAL-AMPH-shock; HAL-AMPH-no shock; and HAL-AMPH-shock.

The immunologic parameters that had been shown to be impaired for the treatment, specifically the percentage of T-lymphocytes and DTH reaction, were evaluated in all the animals as described in Experiment 1. Because similar values of DTH reaction were obtained regardless of the anesthesic used, ether or halothane, we only showed values obtained under ether anesthesia (Fig. 4).

*Statistical Analysis*

Data in Experiment 1 were analyzed with a two-way ANOVA (chronic drug treatment $\times$ shock status). There

were two levels for the chronic drug treatment factor (AMPH or SAL) and two levels for the shock status factor (shock or no shock). In Experiment 2, data were analyzed with a three-way ANOVA (HAL pretreatment × chronic drug treatment × shock status). There were two levels for HAL pretreatment factor (HAL or VEH), two levels for the chronic drug treatment factor (AMPH or SAL), and two levels for the shock status factor (shock or no shock). Following significance in the overall ANOVA, post-hoc comparisons among means were performed with the Newman–Keul's test (the level of significance was set at $p < 0.05$). Values corresponding to the percentages of T- and B-lymphocytes were subjected before the statistical analysis to the following transformation: arc sin $(\%/100)^{1/2}$. Serum hemagglutinin titers were expressed in $\log_{10}$ units of the reciprocal of the antibody titer for statistical analysis.

## RESULTS

### *Experiment 1: Effects of Foot Shock Exposure on Immunologic Parameters in Animals Previously Submitted to Chronic AMPH-Treatment*

Exposure to foot shock resulted in an immunosuppressive effect in animals previously subjected to chronic AMPH-treatment, relative to their VEH-treated controls. This effect was evidenced by a reduced percentage of T-lymphocytes (Fig. 1) and a decreased DTH reaction (Fig. 2). A two-way ANOVA (chronic drug treatment × shock) for T-lymphocytes data indicated a statistically significant effect of chronic drug treatment, $F(1, 20) = 50.97$, $p < 0.01$, a statistically significant effect of shock, $F(1, 20) = 54.11$, $p < 0.01$, and a statistically significant chronic drug treatment × shock interaction, $F(1, 20) = 33.77$, $p < 0.01$. Newman–Keul's post-hoc comparisons among means revealed that exposure to foot shock reduced the percentage of T-lymphocytes in peripheral blood of rats previously exposed to a repeated AMPH administration, compared to the remaining experimental groups. Foot shock exposure did not modify the percentage of T-lymphocytes in animals chronically injected with SAL. No significant difference was observed in the percentage of T-lymphocytes in AMPH-treated animals that were not shocked, compared to SAL-treated rats regardless of their shock exposure.

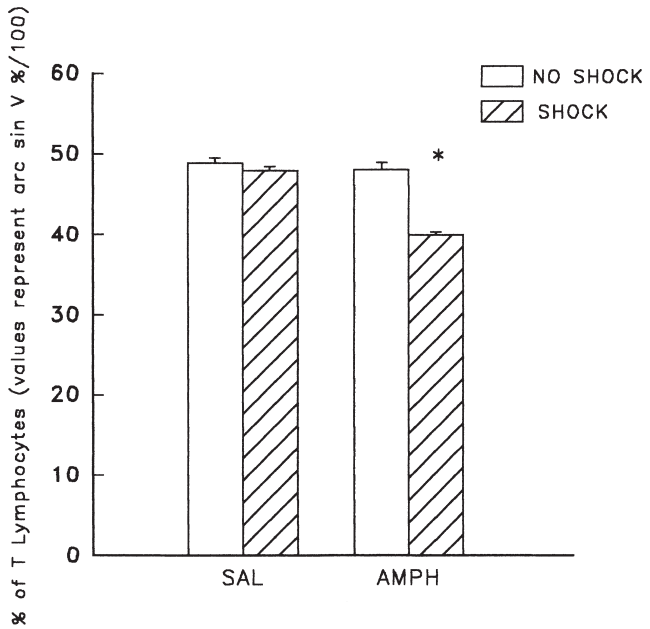


FIG. 1. Effect of foot shock on the percentage of T-lymphocytes in animals previously subjected to a chronic AMPH regimen. The percentage of T-lymphocytes was determined immediately after the foot shock session. Values represent means [arc sin $(\%/100)^{1/2}$ ] + SEM ($n$ = 7–9 for each group). *Significantly different from its control ($p < 0.05$, Newman–Keul's post hoc test).

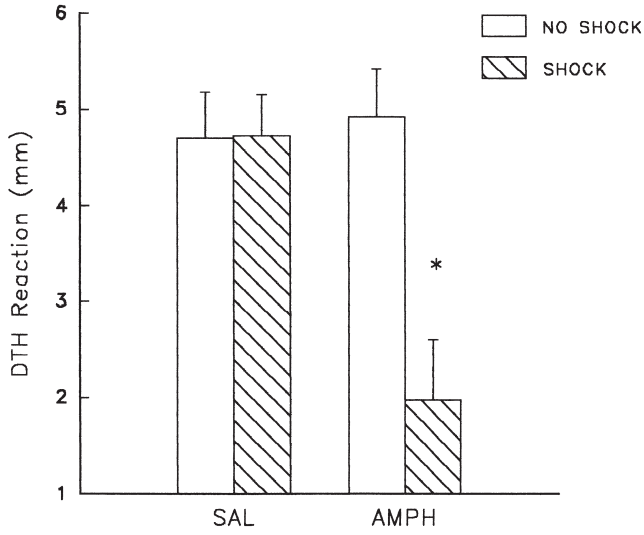


FIG. 2. Effect of foot shock on DTH reaction in animals previously subjected to a chronic AMPH regimen. Values represent means + SEM ($n$ = 7–9 for each group). *Significantly different from its control ($p < 0.05$, Newman–Keul's post-hoc test).

Figure 2 depicts the effect of foot shock stress on the DTH reaction, in rats previously treated with chronic AMPH. A two-way ANOVA revealed a statistically significant chronic drug treatment effect, $F(1, 20) = 6.17$, $p < 0.05$, a statistically significant shock effect, $F(1, 20) = 8.07$, $p = 0.01$, and a statistically significant chronic drug treatment × shock interaction, $F(1, 20) = 8.44$, $p < 0.01$. Newman–Keul's post-hoc analysis demonstrated that the foot shock session induced a significant decrease in the DTH reaction in animals previously submitted to chronic AMPH-treatment, compared to all the other groups ($p < 0.05$). Foot shock did not modify the DTH reaction in animals chronically injected with SAL. No difference in the DTH reaction was observed between unstressed rats subjected to either chronic treatment with AMPH, or to chronic treatment with SAL.

The percentage of B-lymphocytes in peripheral blood as well as the hemagglutinin titers against SRBC were not modified by chronic AMPH-treatment or by foot shock exposure in any experimental group (data not shown).

### *Experiment 2: Effect of HAL on Stress-Induced Immunosuppression Induced by Chronic AMPH-Treatment*

Animals previously exposed to repeated AMPH displayed a decreased percentage of T-lymphocytes and a significant reduction of the cell-mediated immune response following ex-

posure to foot shock stress (Figs. 3 and 4). Both effects were prevented when AMPH-treated rats were injected with HAL, 30 min prior to each daily AMPH injection. ANOVA (HAL pretreatment × chronic drug treatment × shock delivery) on the T-lymphocyte data, revealed a significant HAL pretreatment effect, $F(1, 40) = 21.32$, $p < 0.001$, a significant chronic drug treatment effect, $F(1, 40) = 12.73$, $p = 0.001$, a significant shock effect, $F(1, 40) = 16.75$, $p < 0.001$; and a highly significant HAL pretreatment × chronic drug treatment × shock interaction, $F(1, 40) = 16.75$, $p < 0.001$. Newman–Keul's post-hoc analysis revealed a clear reduction in the percentage of T-lymphocytes in AMPH-treated rats receiving foot shock, compared to the remaining experimental groups ($p < 0.05$). This effect was not evident in rats that received HAL injections prior to their daily AMPH injections (Fig. 3). No difference was observed among animals submitted to chronic treatment with HAL and then exposed or not to the shock stimulus and its appropriated controls.

ANOVA conducted on data from the DTH reaction experiment revealed a significant chronic drug treatment effect, $F(1, 40) = 4.17$, $p < 0.05$, a significant shock delivery effect, $F(1, 40) = 5.18$, $p < 0.05$, and a highly significant HAL pretreatment × chronic drug treatment × shock interaction, $F(1, 40) = 9.03$, $p < 0.005$. Individual post-hoc comparisons using Newman–Keul's test indicated a significant decrease of the DTH reaction in animals chronically treated with AMPH and subsequently exposed to foot shock stress experience, compared with the remaining groups. This stress-induced immunosuppressive effect in rats chronically injected with AMPH was attenuated when the animals were concurrently treated with HAL (Fig. 4). DTH reactions were not altered in rats chronically treated with HAL, regardless of their foot shock exposure. As well, rats receiving HAL pretreatment followed by AMPH treatment in the absence of foot shock exposure, failed to display an altered DTH response relative to control rats.

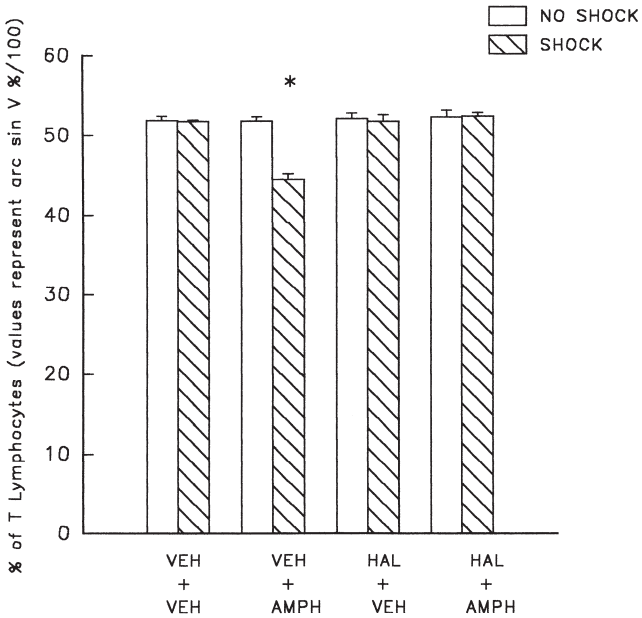


FIG. 3. Effect of foot shock on the percentage of T-lymphocytes in animals previously subjected to a chronic HAL and/or AMPH regimen. Rats in the HAL-AMPH group received HAL pretreatment 30 min preceding the daily AMPH injections. The percentage of T-lymphocytes was determined immediately after the foot shock session. Values represent means [arc sin $(\%/100)^{1/2}$] + SEM ($n = 7$–9 for each group). *Significantly different from its control ($p < 0.05$, Newman–Keul's post-hoc test).

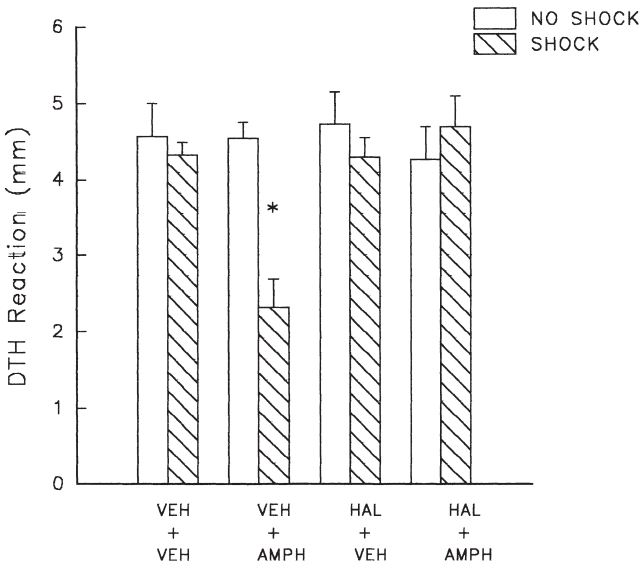


FIG. 4. Effect of foot shock on DTH reaction in animals previously subjected to a chronic HAL and/or AMPH regimen. Rats in the HAL-AMPH group received HAL pretreatment 30 min preceding the daily AMPH injections. *Significantly different from its control ($p < 0.05$, Newman–Keul's post-hoc test).

## DISCUSSION

The present findings demonstrated that repeated administration with a psychomotor stimulant leads to modifications of the immunologic response to a subsequent stressor (foot shock); which did not have any effect by itself on the immune parameters evaluated in the present study. After a 3-day withdrawal period following repeated AMPH, acute exposure to foot shock stress resulted in a clear decrease of both the percentage of T-lymphocytes, and the DTH reaction, in AMPH-treated animals. In contrast, foot shock stress did not induce any effect in vehicle-treated rats. In accordance with previous neurochemical and behavioral findings in rats subjected to an AMPH-treatment regimen similar to the present regimen (2,22,24,27,37,44), immunologic variables in the current study did not reflect changes during the "steady state" (resting conditions). In fact, immunologic changes were only evident following a subsequent stress challenge. The present findings are consistent with previous evidence from this laboratory and other laboratories, which showed that the magnitude of an animal's response to a single stressor at a behavioral, neurochemical, or immunological level, is critically influenced by its past history of stress and/or drug administration (4,5,8,9). Because behavioral, neurochemical and immune changes can be blocked by the concurrent HAL treatment, it may be that DA neurons are a critical link in these events. The involvement of mesocorticolimbic DA systems in AMPH- and stress-induced psychomotor sensitization suggests that these pathways could also be involved in the changes observed in the immune sys-

tem following chronic AMPH. However, the data presented here do not necessarily rule out the possibility that other DA systems can play a role in the immune deficits observed following chronic AMPH-treatment. Behavioral sensitization is observed following repeated stimulation of DA receptors (27,28,31,33,53). Based on the present findings, it is possible to suggest that the sensitization process involves lasting changes in CNS, that can influence not only the behavioral and neurochemical responses, but also the immunologic functioning. However, further experiments with central HAL administration seem necessary to strenghten the notion of the involvement of a central process in AMPH-induced effects at the immunological level. In addition, the present findings do no discard the participation of peripheral catecholaminergic mechanisms in the effects observed in the present work. Although changes in DA transmission have been extensively described, alterations in other neurotransmitter systems may also play a role in this sensitization process.

It might be argued that because the half-life of HAL is very long, accumulation of the drug following the chronic administration might occur (41). However, the lack of effect in animals chronically treated with HAL compared to saline-treated animals indicates that any pharmacokinetic effect that may have occurred, did not have a functional relevance in these studies. Previous findings have described that brief treatments (i.e., 4 days) with HAL or SCH 23390 enhanced the locomotor-stimulating effects of a subsequent psychostimulant challenge (38). Likewise, it has been reported that four daily treatments with a $D_1$ agonist decreases the subsequent response to the locomotor-activating effects of cocaine (3). Although DA-antagonist treatments appear to increase sensitivity to the locomotor effects of psychostimulants, in the present study, pretreatment with HAL did not affect immune functioning following foot shock. Furthermore, HAL blocked the immunosuppressive effect of stress in rats submitted to AMPH treatment. These results suggest that the development of immune sensitization and behavioral sensitization do not necessarily involve the same neurochemical mechanisms, and others neurotransmitter systems may also participate.

The immunosuppressive effect observed following chronic AMPH-treatment in foot shock stress-treated rats is unlikely to be explained by an altered pain threshold, because identical foot shock stress-induced analgesia was observed in animals submitted to the tail flick test, regardless of their exposure to chronic AMPH- or chronic vehicle-treatment (data not shown). Because the results on immune function following chronic AMPH- and/or HAL-treatment were observed regardless of the anesthesic used, ether or halothane, a potential confounding effect of ether on the parameters studied can be discarded. With regard to the humoral immune response assessed in the present study, neither acute exposure to foot shock stress, nor chronic AMPH, had a readily discernible influence on that response, as evidenced by the lack of change in the percentage of B-lymphocytes and in the hemagglutinin titer against SRBC. In addition, previous studies in this laboratory did not reveal a clear stress-induced immunosuppressive effect upon the humoral immunity following exposure to a similar shock experience (4,5). Consistent with these data, Jessop et al. (1986) (26) also reported that foot shock itself had no effect on the humoral response elicited by SRBC injection. However, a reduced level of antibodies following an uncontrollable shock experience was described in another report (34). In this latter study, Laudenslager et al. (1988) (34), used Sprague–Dawley rats and those animals were immunized with keyhole limpet hemocyanine rather than with SRBC. It may be possible that differing rat strains and antigens between the Laudenslager et al. (34) findings and the present findings could explain the discrepancies. Because discrepant data exist in the literature regarding the influence of stress on the humoral immune response, further studies are necessary to clarify this issue.

Repeated exposure to AMPH or other psychostimulants in humans induces various effects, including the development of a psychotic syndrome indistinguishable from paranoid schizophrenia (19,36). In animals, repeated administration of AMPH results in a progressive enhancement of stereotypy and locomotor behavior. The similarity between the paranoid symptoms in humans and the sensitized response in animals has led to the proposal that psychostimulant-induced behavioral sensitization may represent an animal model of paranoid psychosis (43,46). Acute psychotic states can also be induced by stress in psychostimulant abusers and in schizophrenic patients (13,45). In addition, several abnormalities in the immune system have been reported in schizophrenia (21); therefore, and based on the present results, it seems likely that both phenomenon may be linked.

Finally, there is much behavioral and neurochemical evidence demonstrating an interchangeability between AMPH and stress (1,2,22,37). For instance, rats previously sensitized to AMPH are more sensitive to the behavior-activating effect of a subsequent tail-pinch stress (2), and show enduring changes in the response of dopamine neurons, and in pituitary function to a subsequent stress exposure (12,27,44). The present immunologic findings also suggest that repeated administration to AMPH produces hypersensitivity to a subsequent challenge with an environmental stimulus (foot shock), capable of producing a "stress response." Both AMPH and stressful stimuli activate the pituitary–adrenal system, as indicated by increased corticosterone and catecholamine plasma levels (1,30). Furthermore, both stress and chronic AMPH increased the utilization/release of dopamine in mesotelencephalic and hypothalamic dopamine systems (11,18,20,25). Several studies have shown that pretreatment with dopamine receptor antagonists completely blocked the development of behavioral sensitization to the behavioral stimulating effect of AMPH (27,28,31,33,53). In the present work, HAL administered prior to AMPH during the chronic treatment regimen prevented foot shock stress-induced immunosuppression, suggesting a role for dopamine receptors in the modulation of foot shock-induced immunosuppression. It has been reported that the CNS is able to influence the functioning of the immune system through its various neuropeptides, neurohormones, and neurotransmitters (7,16,17). Therefore, the activation of central DA pathways may be implicated in the augmented susceptibility to stress-induced immunodeficiency following repeated AMPH treatment. It is possible to speculate that the sensitized hypothalamic DA system that normally exerts a tonic inhibition of prolactin release, which has immunoenhancing properties and is necessary for normal immune function and resistance to infection (7), contributes to the foot shock stress-induced immunosuppression after chronic AMPH treatment. Behavioral sensitization to AMPH has been consistently demonstrated following treatment with AMPH, morphine, intraventral tegmental area opiate injections, and stress, as well as following combinations of those treatments. In addition, other neuromodulators released in response to stress, such as hypothalamic CRF, endorphins, catecholamines, and corticosterone, could be involved in the sensitization process to AMPH and/or stress. All of those neuromodulators have been demonstrated to be released fol-

lowing aversive stimulation, and are known to possess immunosuppressive properties [see, for review (7,16,17)].

These present data also raise the possibility that enduring changes in central DA systems induced by repeated exposure to psychostimulants could contribute to the development of stress-precipitated immunological disturbances. Considering that drugs of abuse, stress, and psychiatric disorders are all associated with an increased susceptibility to infectious diseases and deficits in the immune system, all of those factors together can be important to contributing causes in the etiology of abnormal immunologic function commonly observed in human addicts. Further studies are needed to identify the neurochemical mechanisms of psychostimulant- and stress-induced alterations in immune function, and the relevance of those alterations in drug abuse and other psychiatric disorders.

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