Pharmacology Biochemistry & Behavior, Vol. 36, pp. 645–651. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Evidence That Shock-Induced Immune Suppression Is Mediated by Adrenal Hormones and Peripheral β-Adrenergic Receptors

JOAN E. CUNNICK, DONALD T. LYSLE, BARBARA J. KUCINSKI AND BRUCE S. RABIN

Division of Clinical Immunopathology, Department of Pathology and the Brain, Behavior and Immunity Center
Presbyterian-University Hospital, Desoto at O'Hara Streets
University of Pittsburgh, PA 15213-2582

Received 26 February 1990

CUNNICK, J. E., D. T. LYSLE, B. J. KUCINSKI AND B. S. RABIN. *Evidence that shock-induced immune suppression is mediated by adrenal hormones and peripheral β-adrenergic receptors.* PHARMACOL BIOCHEM BEHAV **36**(3) 645–651, 1990.—Our previous work has demonstrated that presentations of mild foot-shock to Lewis rats induces a suppression of splenic and peripheral blood lymphocyte responses to nonspecific T-cell mitogens. The present study demonstrated that adrenalectomy prevented the shock-induced suppression of the mitogenic response of peripheral blood T-cells but did not attenuate the suppression of splenic T-cells. Conversely, the beta-adrenergic receptor antagonists, propranolol and nadolol, attenuated the shock-induced suppression of splenic T-cells in a dose-dependent manner but did not attenuate suppression of the blood mitogen response. These data indicate that distinct mechanisms mediate the shock-induced suppression of T-cell responsiveness to mitogens in the spleen and the peripheral blood. The results indicate that the peripheral release of catecholamines is responsible for splenic immune suppression and that adrenal hormones, which do not interact with β-adrenergic receptors, are responsible for shock-induced suppression of blood mitogenic responses.

Immunosuppression Stress Propranolol Nadolol Beta-adrenergic Adrenalectomy Glucocorticoids Lymphocytes Concanavalin A Phytohemagglutinin

IT is well documented that stress can produce alterations in the responsiveness of the immune system. In humans, bereavement resulting from the death of a spouse is accompanied by a decreased mitogen response (2) and the stress of test taking in medical students is correlated with decreases in mitogen responsiveness, interleukin-2 (IL-2) production and natural killer cell (NK) function (12). In animals, surgical stress can reduce NK cell function (22,23), the stress of electric foot-shock can suppress mitogen responsiveness (18), and restraining the movement of an animal can depress delayed type hypersensitivity, NK activity and phagocytic activity (21).

We have previously demonstrated that different types of stressors can produce similar and consistent changes in the functional response of the immune system in rats. These stressors (electric foot-shock, 2-deoxy-D-glucose, and conditioned fear/anxiety) are each capable of suppressing the lymphocyte response to T-cell mitogens (15–17). The T-lymphocytes in various immune compartments are differentially affected by these stressors, as demonstrated by different rates for recovery of function after stressor exposure and the ability of spleen lymphocytes to habituate to several days of stress (i.e., after 5 days of stressor exposure splenic lymphocytes respond normally to T-cell mitogens), while blood lymphocytes do not (17). These findings have led us to suggest that different mechanisms are acting to suppress the response of T-lymphocytes in the blood and in the spleen.

There have been few in vivo studies of the mechanisms responsible for the stress-induced changes of immune response. Shavit and colleagues (27) have demonstrated that opiates are involved in the stress-induced suppression of NK activity. They were able to block stress induced NK suppression by administration of naloxone. Weber and Pert (29) have recently demonstrated that opiates are acting to suppress NK activity at the level of the CNS in the periaqueductal gray matter. Although opiates are involved in the shock-induced suppression of splenic NK activity, our laboratory has demonstrated that they are not involved in suppression of the T-cell mitogenic responsiveness of lymphocytes in the blood or spleen, as pretreatment of rats with naltrexone was not capable of preventing the shock-induced suppression (7).

Other mediators of immune suppression have been identified but there exist many conflicting reports as to their role in vivo. Adrenocorticotropic hormone (ACTH) and glucocorticoids, released from the adrenal gland in response to ACTH, have been

shown to be immunosuppressive to lymphocyte responses in vitro (6,19). However, others have not been able to correlate stress-related suppression of immune responses with increased levels of glucocorticoids (2). Furthermore, Miller and colleagues at Mount Sinai Medical Center have noted differential expression of glucocorticoid receptor subtypes in immune compartments. Whereas spleen lymphocytes exhibit both Type I and Type II glucocorticoid receptor binding, the thymic lymphocytes exhibit Type II binding only (personal communication). This data indicates that different immune compartments may be differentially responsive to glucocorticoids. The mitogenic response of lymphocytes in vitro can be inhibited directly by the addition of catecholamine agonists such as isoproterenol and norepinephrine (10). This inhibition is mediated by β-adrenergic receptors on the surface of the lymphocytes (4,14). Stress induces an increased turnover of catecholamines in the brain and a discharge of catecholamines by sympathetic neurons in the periphery (13). Felten and colleagues (9) have shown that the spleen contains sympathetic innervation which terminates in lymphocyte areas of the spleen. However, there is no direct evidence that catecholamines are inducing immunologic changes during stress in vivo.

The purpose of the present research was to examine the role of adrenal hormones and the sympathetic release of catecholamines in our foot-shock-induced immune suppression of mitogenic responses. To this end, adrenalectomized rats were used to determine if adrenal hormones were involved in suppression of the blood, and spleen mitogenic response to concanavalin A (Con A). In addition, the β-adrenergic receptor antagonists propranolol and nadolol were used to block the binding of catecholamines to their receptors and to determine if the antagonist could attenuate the suppression of shock-induced suppression of blood and spleen mitogenic responses. As nadolol does not cross the blood-brain barrier (1), intraperitoneal (IP) administration of the drug was used to specifically determine the role of peripheral β-adrenergic receptors.

METHOD

Animals

Male rats of the Lewis strain, 65 days old and 250–300 grams in weight, were purchased from Charles River Laboratories. For the adrenalectomy experiments, animals were adrenalectomized or sham adrenalectomized by Charles River Laboratories and shipped 10 days after surgery along with unoperated control animals of the same age. Upon arrival, the subjects were individually caged in a colony room where a reversed day-night (12-hour) cycle was maintained through artificial illumination. They received free access to both food (Wayne Lab Blox) and water (0.9% saline for the adrenalectomized rats) throughout the experiment, and were acclimated to the vivarium for two weeks prior to the experimental manipulations.

Shock Apparatus

Eight identical rodent chambers (Coulbourn Instruments Model E10-10), measuring $25 \times 30 \times 33$ cm, served as the shock apparatuses. The chambers had clear Plexiglas side walls, sheet-metal top and end walls, and a grid floor consisting of bars 0.24 cm in diameter, spaced 0.87 cm apart. An 8.5-cm, 3.5-ohm speaker (Quam model 3A05), externally mounted on the wall of each chamber, was connected to an audio generator (BRS/LVE Model AU-902) to provide an auditory signal: a 78-dB (re 0.0002 dyne/cm^2) clicking sound that connected through timer circuitry to the output of a shock generator and scrambler (BRS/LVE Models 903 and SC 902) to provide a 5.0-second, 1.6-mA, foot-shock. The chambers were individually housed in identical sound-attenuating cubicles, $50 \times 60 \times 88$ cm, that were located in a room adjacent to the programming equipment. A 100-W, 120-V bulb, recessed behind a frosted plate in the ceiling of each cubicle, was operated at 85 V, AC, to provide diffuse illumination of the chamber. An ambient sound level of 72 dB was provided by operating the cubicle's ventilating fan at 57 V, AC.

Parameters of Shock

The experimental subjects were presented with 16 signaled-shocks in a single 64-min session. The session began one hour into the dark phase of the day-night cycle. For all groups, the shocks were delivered on a variable basis, every 2–6 minutes, with the time between shocks averaging 4 minutes. Each shock was preceded by a 15-second clicker signal which coterminated with the shock. Control subjects were handled and transported in a manner identical to the experimental subjects, but were not placed in the shocking apparatus.

Immediately following the last shock experience, each experimental subject was rapidly sacrificed by cervical dislocation with a clamp. The control subjects were transported from their home cages to the chamber room, and sacrificed in the same manner and at the same time as the experimental subjects. Blood was collected via the abdominal aorta into 5-ml heparinized syringes, the spleen was placed in a 15-ml polypropylene centrifuge tube containing RPMI-1640 media and all samples were transported to the tissue-culture laboratory for preparation.

Adrenalectomy experiment. To determine the role of glucocorticoids in shock-induced immune suppression, adrenalectomized (ADX), sham-adrenalectomized (SHAM) or unoperated (NORMAL) male rats were evaluated. Subjects were randomly assigned to two groups ($n = 10$). One group received a single, 64-min session of 16 signaled foot-shocks. The other group was not exposed to the stressor and remained in their home cages as controls. The experiment was performed in two replicates and the data were combined for analyses. Spleen and blood leukocytes were enumerated and tested for responsiveness to the T-cell mitogen, Con A (Difco). To test for the completeness of adrenalectomy, plasma samples were assayed for corticosterone. The error for the corticosterone radioassay is 0.2 μg/dl and values below this level, as in the ADX groups, can be interpreted as a lack of corticosterone. It should be noted that one animal in the shocked/ADX group was eliminated due to the detection of corticosterone (>1.0 μg/dl) in the plasma. The results for this subject were eliminated from all data sets.

Treatment with receptor antagonists. Propranolol hydrochloride (Ayerst Laboratories), a β-adrenergic receptor antagonist, was diluted in PBS (pH 7.2) and administered at 0.5, 1.0 or 2.0 mg/kg. Nadolol (a gift from E. R. Squibb & Sons), a form of propranolol (cis-5-[3-[(1,1-dimethylethyl)amino]-2-hydroxy-propoxy]-1,2,3,4-tetrahydro-2,3-naphthalenediol) which does not cross the blood-brain barrier, was dissolved in DMSO, diluted in PBS and administered at 0.5, 1.0 or 2.0 mg/kg. The vehicle control for the receptor antagonists was an equi-volume of PBS or PBS/2% DMSO, as was appropriate. Thirty minutes prior to the shock session, experimental animals received an intraperitoneal (IP) injection of one of the drug doses or the vehicle. Injection control rats received similar injections but remained in their cages for 90 min, a time equal to the shock session plus the 30-min postinjection period. After the shock session or 90-min time period, the experimental, injection control, and noninjected control rats, were sacrificed and the blood and spleens were collected.

Mitogen Assay

Spleens were dissociated to a single cell suspension. Splenic

leukocytes were enumerated using a Coulter Counter (Model ZBI) and diluted to 5×10^6 leukocytes/ml. Leukocytes per ml of whole blood were determined using a Unopette and hemocytometer. A mitogen stimulation assay was performed as previously described (17) with whole spleen suspension or whole blood (diluted 1:10 with supplemented media containing heparin, 5 Units/ml), and Con A at concentrations ranging from 0.5–10 μg/ml. Con A at a final concentration of 5.0 μg/ml for the whole spleen cultures and 10 μg/ml for the whole blood cultures were the optimal concentrations of mitogen (i.e., provided maximal lymphocyte stimulation for the control samples). The spleen cultures were pulsed with 1 μCi ^{3}H-thymidine (specific activity 6.7 Ci/mmol; Dupont-New England Nuclear) in 50 μl of RPMI-1640 during the last 5 hours of a 48-hour incubation. The cultures were harvested onto glass filter paper using a Skatron Cell Harvester (Skatron Inc.) and the incorporation of ^{3}H-thymidine was determined with a liquid scintillation counter (Packard Model 1500) and was expressed as counts per minute (cpm). The blood leukocyte cultures were incubated for a total of 96 hours and were pulsed with ^{3}H-thymidine (1 μCi/well) for the last 18 hours of the incubation. The cultures were harvested and counted in the same manner as the spleen cultures. The average cpm/well for the blood cultures were normalized to cpm per 10^5 leukocytes.

Plasma corticosterone assay. A sample of the heparinized blood obtained from the abdominal aorta was centrifuged at 12000 rpm for 3 minutes, and 0.5 ml of plasma was removed and frozen at −70°C. Plasma corticosterone was determined in the laboratory of Dr. S. Antelman (Department of Psychiatry, University of Pittsburgh) by a competitive protein binding radioassay (20). This assay requires only 25 μl of plasma and is sensitive to 0.2 μg/dl.

Statistical Treatment of Data

A computerized program for analysis of variance (Statistix, NH Analytical Software) was used to assess differences among experimental and control groups. For the adrenalectomy experiment, the optimal cpm of the mitogenic response for blood and spleen were separately analyzed using a standard two × three analysis of variance. One factor consisted of treatment, a session of signaled-shock or home cage control. The second factor was operation, adrenalectomy (ADX), sham operated or unoperated.

For the experiments in which a receptor antagonist was administered, the cpm of the mitogenic response for blood and spleen were separately analyzed using a standard analysis of variance which took the form of a two × four plus one design. One factor was treatment, a session of signaled-shock or home cage control; the second factor was injection, vehicle or drug (low, medium or high dose); and the additional group was the noninjected home cage control.

For each of the experiments, the total leukocytes per spleen and per ml whole blood were also analyzed. The significance level for all analyses was set at a probability of less than or equal to 0.05.

RESULTS

Adrenalectomy Experiment

The results of the assay for plasma corticosterone are presented in Table 1. An analysis of variance demonstrated a significant increase in corticosterone due to treatment, $F(1,39) = 622.11$, $p<0.0001$, and a significant main effect of the operation, $F(2,39) = 287.18$, $p<0.0001$. Orthogonal contrasts of operation demonstrated that the corticosterone levels of the sham-operated groups were significantly elevated in comparison to the normal groups, $F(1,39) = 5.06$, $p<0.04$. In contrast, the levels in the adrenalectomy (ADX) groups were significantly suppressed in comparison to the normal groups, $F(1,39) = 381.81$, $p<0.0001$.

TABLE 1

CORTICOSTERONE LEVELS IN ADRENALECTOMIZED, SHAM-OPERATED OR UNOPERATED, NORMAL RATS WHICH WERE SHOCKED OR REMAINED IN THEIR HOME CAGES (CONTROL)

	Control	Shocked
ADX	0.11 ± 0.08*	0.02 ± 0.02
SHAM	5.09 ± 1.83	36.27 ± 2.30
NORMAL	5.19 ± 2.21	31.92 ± 1.35

*Mean ± SE (μg/dl).

The analysis showed a significant interaction of treatment and operation, $F(2,39) = 159.73$, $p<0.0001$. Orthogonal contrasts demonstrated a significant interaction of the sham-operated groups with the normal groups, $F(1,39) = 5.52$, $p<0.25$, as well as a highly significant interaction of the ADX groups and the normal groups, $F(1,39) = 200.79$, $p<0.0001$. This indicates that the shocked/sham-operated group had a significantly higher level of corticosterone than the shocked/control group, whereas the ADX groups lacked corticosterone.

The replication factor for the corticosterone levels showed no significant main effect, but did significantly interact with treatment and as well as the interaction of treatment and operation, $F(1,39) = 43.28$, $p<0.0001$, and $F(2,39) = 11.85$, $p<0.001$, respectively. These interactions were due to a decreased baseline and an increased corticosterone response to stress of the sham-operated and normal groups during the first replication.

Thus, the adrenalectomy operations were highly effective in eliminating baseline levels of corticosterone and preventing the normal stress-induced rise in corticosterone levels. Futhermore, the sham operation appeared to sensitize the shocked rats to release a greater quantity of corticosterone.

The results of the blood mitogenic response to the optimal concentration of Con A are illustrated in Fig. 1. The 2×3 analysis of variance for the blood response to the optimal concentration of mitogen showed a significant main effect for treatment, $F(1,39) = 60.03$, $p<0.0001$, indicating that shock induces a suppression of the mitogenic responsiveness of blood lymphocytes. The analysis of variance also demonstrated a significant main effect of operation for these groups, $F(2,39) = 14.78$, $p<0.0001$. Planned orthogonal contrasts for this effect revealed that there was no difference between the normal and sham-operated rats. However, these two groups were significantly suppressed in comparison to the adrenalectomized groups, $F(1,39) = 28.94$, $p<0.0001$. Most importantly, the interaction term for the analysis of treatment and operation was significant, $F(2,39) = 3.26$, $p<0.05$. The orthogonal contrasts revealed that the interaction of the normal and sham operated groups was not significant, but the interaction of these groups and the ADX groups was significant, $F(1,39) = 5.76$, $p<0.025$. Therefore, these data demonstrate an attenuation of shock-induced immune suppression in rats lacking adrenal glands and the ability to produce corticosterone. There were no significant main effects or interactions for the replication factor in the results of the whole blood mitogen assay.

Figure 2 displays the results of the mitogenic response of spleen lymphocytes to the optimal concentration of Con A. The analysis of variance of this data revealed a significant main effect of treatment, $F(1,39) = 188.92$, $p = 0.0001$. This demonstrates that foot-shock can induce a pronounced decrease in the mitogenic response of splenic lymphocytes.

The analysis of variance also demonstrated a significant main

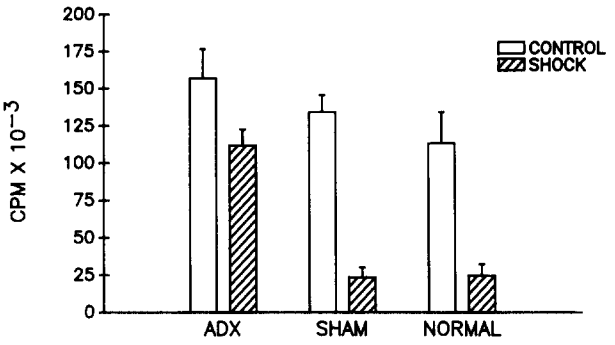

FIG. 1. Proliferative response of whole blood lymphocytes to 10 μg/ml Con A from adrenalectomized, sham-operated and normal rats. Shocked rats received 16 electric, foot-shock presentations and controls remained undisturbed in their home cages. Results are mean ± SE.

effect of operation, $F(2,39) = 3.71$, $p<0.04$. Contrasts revealed that the sham-operated group was significantly suppressed compared to the normal, unoperated groups, $F(1,39) = 6.3$, $p<0.02$. However, the ADX groups were not significantly different from the normal, unoperated groups. The interaction term for treatment and operation was also significant, $F(2,39) = 4.29$, $p<0.03$. Contrasts revealed that the interaction of normal groups and sham-operated groups was significant, $F(1,39) = 7.4$, $p<0.01$, indicating a greater suppression of the mitogenic response in the shocked/sham-operated group. However, the ADX groups did not significantly interact with the unoperated (normal) control groups, indicating that the shock-induced suppression of the splenic mitogen responses of these two groups was equivalent. Furthermore, an ancillary contrast revealed a significant interaction of the ADX groups and the sham-operated groups, $F(1,39) = 5.34$, $p<0.03$, indicating a greater suppression of mitogenic response in the shocked/sham-operated group.

These results indicate that adrenalectomy did not attenuate the shock-induced immune suppression of splenic lymphocytes. Furthermore, the sham operation procedure enhanced the shock-induced suppression of the mitogenic response of splenic lymphocyte.

For the splenic response to the optimal concentration of Con A, the replication factor was significant, $F(1,39) = 52.09$, $p<0.0001$, and significantly interacted with treatment, $F(1,40) = 15.01$, $p<0.001$. The suppression was of similar magnitude for each replication (35% and 38% of the control response). However, the

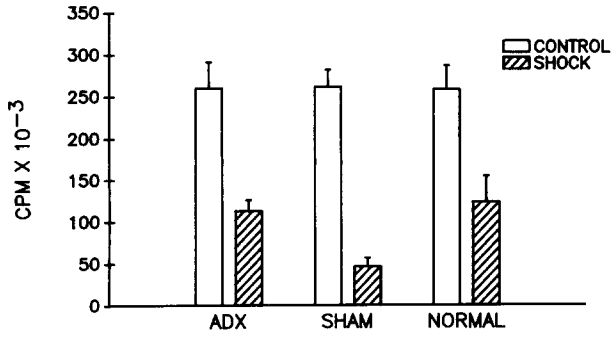

FIG. 2. Proliferative response of whole spleen lymphocytes to 5.0 μg/ml Con A from adrenalectomized, sham-operated and normal rats. Shocked rats received 16 electric, foot-shock presentations over 64 min and controls remained undisturbed in their home cages. Results are mean ± SE.

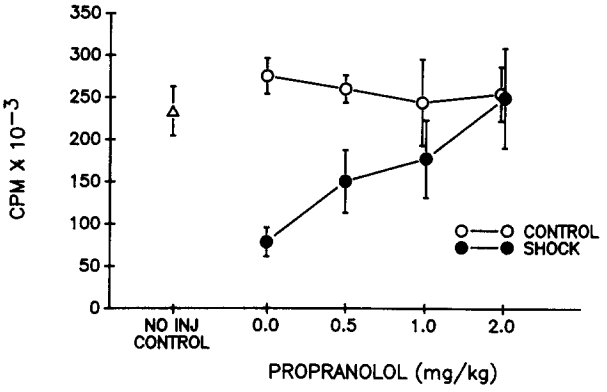

FIG. 3. Proliferative response of whole spleen lymphocytes to 5.0 μg/ml Con A. Rats were injected IP, 30 min prior to stressor exposure and received vehicle (PBS), or 0.5, 1.0 or 2.0 mg/kg propranolol. The nontreated controls received no injections. Shocked rats received 16 electric, foot-shock presentations and controls remained undisturbed in their home cages. Results are mean ± SE.

first replication had a higher baseline response (3.34×10^5 cpm) than the second (1.23×10^5 cpm).

These findings suggest that the adrenal gland hormones are involved in the shock-induced immune suppression observed in the blood lymphocytes. However, adrenalectomy did not have an attenuating effect on the shock-induced suppression of the splenic lymphocyte response. This supports the hypothesis of different mechanisms mediating the suppressive effects of shock in different immune compartments.

*Propranolol experiments.*The results of the spleen mitogenic response to the optimal concentration of Con A are displayed in Fig. 3. Overall analysis of variance of the optimal concentration showed a significant difference among the groups, $F(8,45) = 3.03$, $p<0.01$. A contrast of the no injection control group with the vehicle control group showed no significant effect of the injection procedure.

The analysis demonstrated a significant suppressive effect of the shock treatment, $F(1,45) = 12.89$, $p<0.001$, but no significant main effect of the injections. However, there was a marginally

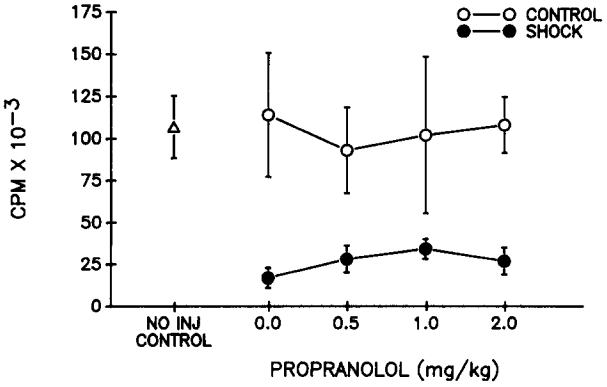

FIG. 4. Proliferative response of whole blood lymphocytes to 10 μg/ml Con A. Rats were injected IP, 30 min prior to stressor exposure and received vehicle (PBS), or 0.5, 1.0 or 2.0 mg/kg propranolol. The nontreated controls received no injections. Shocked rats received 16 electric, foot-shock presentations and controls remained undisturbed in their home cages. Results are mean ± SE.

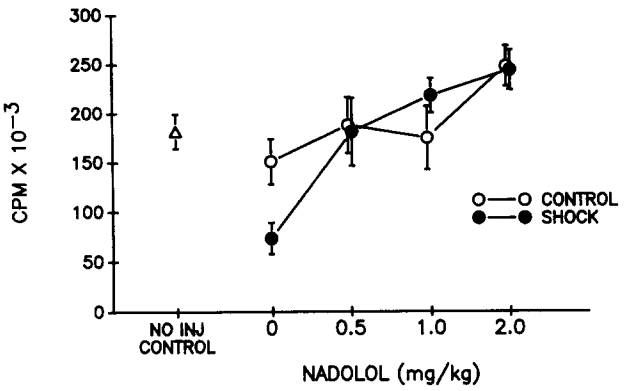

FIG. 5. Proliferative response of whole spleen lymphocytes to 5.0 μg/ml Con A. Rats were injected IP, 30 min prior to stressor exposure and received vehicle (PBS/2% DMSO), or 0.5, 1.0 or 2.0 mg/kg nadolol. The nontreated controls received no injections. Shocked rats received 16 electric, foot-shock presentations and controls remained undisturbed in their home cages. Results are mean ± SE.

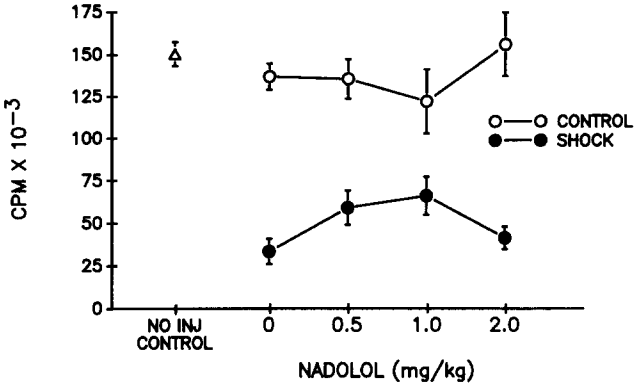

FIG. 6. Proliferative response of whole blood lymphocytes to 10 μg/ml Con A. Rats were injected IP, 30 min prior to stressor exposure and received vehicle (PBS/2% DMSO), or 0.5, 1.0 or 2.0 mg/kg nadolol. The nontreated controls received no injections. Shocked rats received 16 electric, foot-shock presentations and controls remained undisturbed in their home cages. Results are mean ± SE.

significant interaction of treatment with injection, $F(3,45)=2.33$, $p=0.085$. Planned polynomial contrasts revealed a significant linear effect for the interaction, $F(1,45)=6.86$, $p<0.015$. This interaction indicates that propranolol attenuated the stress-induced suppression of the splenic mitogen response in a dose-dependent manner.

The results of the blood mitogenic response to the optimal concentration of Con A are displayed in Fig. 4. Overall analysis of variance showed a significant difference among the groups, $F(8,45)=3.22$, $p<0.006$. Comparison of the no injection control group with the vehicle control group showed no significant effect of the injection procedure.

The analysis demonstrated a significant suppressive effect of the shock treatment, $F(1,45)=22.18$, $p<0.0001$. However, there was no significant main effect of the propranolol injections, nor was there a significant interaction of treatment with injection. These results indicate that administration of propranolol did not attenuate the shock-induced suppression of the blood mitogen response.

These data demonstrate that the immunosuppressive effects of stress in the spleen can be attenuated by propranolol, a β-adrenergic receptor antagonist, in a dose-dependent manner. This finding suggests a role for endogenous β-adrenergic receptor agonists in the mediation of stress-induced suppression of splenic T-cell function. The stress-induced immune suppression of the blood mitogenic response was not affected by the administration of propranolol. Thus, the suppression of spleen and blood mitogenic responses is induced by two distinct pathways.

Nadolol experiments. The results of the spleen mitogen assay to the optimal concentration of Con A is displayed in Fig. 5. Overall analysis of variance showed a significant difference among the groups, $F(8,45)=4.75$, $p<0.001$. Comparison of the no injection control group with the vehicle control group showed no significant effect of the injection procedure.

The analysis did not reveal a significant main effect of the shock treatment. However, an ancillary contrast did reveal that the shocked/vehicle group was significantly suppressed relative to the vehicle control group, $F(1,45)=5.11$, $p<0.03$. The analysis showed a main effect for the injections, $F(3,45)=10.38$, $p<0.001$. Polynomial contrasts demonstrated a significant linear effect of drug dose, $F(1,45)=29.05$, $p<0.0001$. There was not a significant interaction of treatment with injection.

The results of the blood mitogen assay to the optimal concentration of Con A is displayed in Fig. 6. Overall analysis of variance showed a significant difference among the groups, $F(8,45)=17.04$, $p<0.0001$. A contrast of the no injection control group with the vehicle control group showed no significant effect of the injection procedure.

The analysis revealed a significant suppressive effect of the shock treatment, $F(1,45)=107.54$, $p<0.0001$. However, there was not a main effect for injection, nor was there a significant interaction of shock treatment with injection.

Corticosterone assays were conducted on plasma samples and overall analysis of variance indicated a significant effect of the treatments, $F(8,45)=21.4$, $p<0.0001$. All groups which received shock had a marked increase of plasma corticosterone (39.66 μg/dl) in comparison to the control groups (14.36 μg/dl), $F(1,45)=151.53$, $p<0.0001$. There were no other significant contrasts. These findings indicate that the attenuating effects of the β-adrenergic receptor antagonist, nadolol, is not through modulation of corticosterone.

These results indicate that peripheral administration of nadolol, a β-adrenergic receptor antagonist, is highly effective in attenuating the immunosuppressive effects of stress on the splenic T-cell mitogen response. In contrast, the stress-induced immune suppression of the blood mitogenic response was not affected by the peripheral administration of nadolol. This suggests that stress-induced immune suppression of splenic lymphocytes is mediated via peripheral catecholamines binding to β-adrenergic receptors.

Blood and spleen leukocyte counts. Enumeration and analysis of total splenic leukocytes in all experiments showed no significant difference between shocked and control rats in those studies using beta-adrenergic receptor antagonists (8.13×10^8 leukocytes/spleen). However, in the study of adrenalectomized rats, there was a significant leukopenia due to shock treatment (Shock: 7.8×10^8; Control: 8.8×10^8 leukocytes/spleen), $F(1,39)=11.52$, $p<0.01$, as well as a significant leukopenia induced by operation, $F(2,39)=6.84$, $p<0.01$. Orthogonal contrasts demonstrated that the ADX groups had significantly fewer splenic leukocytes than the sham-operated and normal groups, $F(1,39)=12.89$, $p<0.001$ (ADX: 7.7×10^8; Sham: 8.9×10^8; Normal: 8.5×10^8 leukocytes/spleen).

Enumeration and analysis of total blood leukocytes revealed that shock treatment had a significant and consistent effect. In all experiments using beta-adrenergic receptor antagonists, shock

treatment reduced blood leukocyte numbers: propranolol, $F(1,45) = 31.25$; and nadolol, $F(1,45) = 13.88$; $p<0.0001$, for both experiments (means leukocyte numbers: 3.26×10^6/ml for the shocked groups, 5.96×10^6/ml for the control groups). There was no effect of drug on blood leukocyte number for any of the experiments.

For the adrenalectomy experiment, there was also a significant leukopenia in the blood induced by the shock treatments, $F(1,39) = 69.57$, $p<0.0001$. There was no significant effect of operation. However, there was a significant interaction of treatment and operation on blood leukocyte numbers, $F(2,39) = 4.26$, $p<0.025$. Numbers of leukocytes were similar in all control groups and the interaction was due to a greater leukopenia in the shocked/sham-operated and normal groups than in the shocked/ADX group, $F(1,39) = 5.66$, $p<0.025$ (mean leukocytes: 5.12×10^6/ml for all three control groups; 2.48×10^6/ml for shocked/sham and normal groups; and 3.67×10^6/ml for the shocked/ADX group).

These results indicate that the stress of treatment with mild electric foot-shock is capable of leukopenia in a consistent manner. However, this stressor can have variable results on the leukocyte numbers in the spleen. The disparity of changes in leukocyte numbers in the spleen and blood further emphasizes the differences in stress-induced mechanisms of change in these two lymphoid organs.

DISCUSSION

These studies replicate our previous work demonstrating the ability of stressors to suppress blood and splenic T-cell mitogenic responses to Con A (7,17). In addition, the present studies extend that work by demonstrating differential mechanism for induction of immune suppression in the blood and spleen. The shock-induced suppression of whole blood lymphocytes was attenuated in those rats which had been adrenalectomized, but not in rats which received propranolol or nadolol. These findings indicate that the suppression of blood lymphocytes is mediated by an adrenal hormone such as corticosterone.

Although adrenalectomy prevented the majority of blood lymphocyte suppression to Con A, shocked/ADX rats did not respond at the same level as the control/ADX rats. This difference may have been due to stress-induced increases in the plasma levels of hormones not of adrenal origin, such as ACTH. High levels of ACTH can be suppressive to lymphocyte function (6) and could be responsible for the suppression of the blood mitogen response in shocked/ADX rats. ACTH levels are usually controlled by an inhibitory feed-back signal of increased glucocorticoids. However, adrenalectomized rats lack glucocorticoid production and have a higher baseline level of ACTH (8). It might be expected that stress could induce an increase in the level of ACTH over the higher baseline levels (25).

The suppression of the splenic lymphocyte response to Con A could be attenuated by IP administration of either propranolol or nadolol. Both of these drugs are β-adrenergic receptor antagonists. Moreover, nadolol does not cross the blood-brain barrier (1). These findings indicate that shock-induced immune suppression of splenic lymphocytes is modulated by the release of epinephrine or norepinephrine in the periphery. That the adrenal medullary catecholamines are not involved is evidenced by the lack of effect of adrenalectomy on the splenic response to shock. A likely source of the endogenous β-adrenergic ligand is the sympathetic noradrenergic innervation of this immune organ. The effect of neuronal norepinephrine on the T-lymphocytes could be direct. T-Lymphocytes have been demonstrated to possess β-adrenergic receptors which induce an increase in cAMP when stimulated (3,5). Increased cAMP is known to inhibit the second messenger system which mediates lymphocyte activation via the T-cell receptor.

The augmentation of immune suppression of splenic lymphocytes in shocked/sham-operated rats is intriguing and may be due to an interaction of glucocorticoid and catecholamine release. Glucocorticoids can alter the density of β-adrenergic receptors on several cell types making them more sensitive to the effects of norepinephrine (26,28). The stress of the sham operation and resultant release of glucocorticoids may be involved in the sensitization of the splenocytes from shocked/sham-operated rats to the peripheral release of catecholamines.

Leukopenia was found in the blood of all shocked rats and has been reported by other investigators (11). However, we have not found changes in the percentages of T-lymphocyte populations in the spleen or peripheral blood of shocked rats (unpublished observation). This indicates a functional change in lymphocyte responsiveness due to endogenous mediators.

In studies using humans exposed to stressors such as test-taking or bereavement, there is an observation of suppressed mitogenic responsiveness of peripheral blood leukocytes (2,12). However, these studies have not been able to correlate the decreased mitogenic responsiveness with increased levels of serum glucocorticoids. This is not surprising as glucocorticoid levels can return to normal levels in hours and changes in circulating concentrations of adrenal steroids may easily be missed. Furthermore, the effects of glucocorticoids on blood lymphocytes appear to be long lasting. Previous studies (17) indicate that the shock-induced immune suppression of blood lymphocytes in rats does not return to normal levels until some time between 48 and 96 hours after a single session of 16 foot-shocks.

Taken together, these studies indicate separate pathways of induction of immune suppression in blood and spleen lymphocytes and represent the first in vivo indication of the differential mechanisms that are operational in stress-induced immune changes. These mechanisms associated with shock-induced immune suppression may be operational with other stressors which cause immune suppression, as all stressors which we have studied present similar patterns of immune changes.

ACKNOWLEDGEMENTS

The authors would like to thank Ada Armfield and Paul Wood for their technical support, Matthew Fraser for his editorial assistance and Harry Fowler for the use of his animal facilities and shock apparatus. Support for this research is through NIMH Grants MH43411 and MH46284 and the Pathology Education Research Foundation.

REFERENCES

1. Antonaccio, M. J.; DeForrest, J. Pharmacology of nadolol. In: Gross, E. F., ed. International experience with nadolol, a long-acting β-blocking agent. New York: Academic Press; 1981:11–24.
2. Bartrop, R. W.; Lockhurst, E.; Lazarus, L.; Kiloh, L. G.; Penny, R. Depressed lymphocyte function after bereavement. Lancet 1:834–836; 1977.
3. Bourne, H. R.; Lichtenstein, L. M.; Melmon, K. L.; Henney, C. S.; Weinstein, Y.; Shearer, G. M. Modulation of inflammation and immunity by cyclic AMP. Science 184:19–28; 1974.
4. Brodde, O. E.; Engel, G.; Hoyer, D.; Bock, K. D.; Webber, F. The beta-adrenergic receptor in human lymphocytes—Subclassification by the use of a new radio-ligand (±)-^{125}iodocyanopindolol. Life Sci. 29:2189–2198; 1981.
5. Byron, J. W. Evidence for a β-adrenergic receptor initiating DNA synthesis in haemopoietic stem cells. Exp. Cell Res. 71:228–232; 1972.
6. Clarke, B. L.; Bost, K. L. Differential expression of functional adrenocorticotropic hormone receptors by subpopulations of lympho-

cytes. J. Immunol. 143:464–469; 1989.
7. Cunnick, J. E.; Lysle, D. T.; Armfield, A.; Fowler, H.; Rabin, B. S. Shock-induced modulation of lymphocyte responsiveness and natural killer activity: Differential mechanisms of induction. Brain Behav. Immun. 2:102–113; 1988.
8. Dallman, M. F.; Akana, S. F.; Jacobson, L.; Levin, N.; Cascio, C. S.; Shinsako, J. Characterization of corticosterone feedback regulation of ACTH secretion. Ann. NY Acad. Sci. 512:402–414; 1987.
9. Felten, D. L.; Felten, S. Y.; Bellinger, D. L.; Carlson, S. L.; Ackerman, K. D.; Madden, K. S.; Olschowki, J. A.; Livnant, S. Noradrenergic sympathetic neural interactions with the immune system: Structure and function. Immunol. Rev. 100:225–260; 1987.
10. Goodwin, J. S.; Messner, R. P.; Williams, R. C. Inhibitors of T-cell mitogenesis: Effect of mitogen dose. Cell. Immunol. 45:303–308; 1979.
11. Keller, S. E.; Weiss, J. M.; Schleifer, S. J.; Miller, N. E.; Stein, M. Stress-induced suppression of immunity in adrenalectomized rats. Science 221:1301–1304; 1983.
12. Kiecolt-Glaser, J. K.; Garner, W.; Speicher, C.; Penn, G. M.; Holliday, J.; Glaser, R. Psychosocial modifiers of immunocompetence in medical students. Psychosom. Med. 46:7–14; 1984.
13. Kopin, I. J.; Eisenhofer, G.; Goldstein, D. Sympathoadrenal medullary system and stress. In: Chrousos, G. P.; Loriaux, D. L.; Gold, P. W., eds. Mechanisms of physical and emotional stress. New York: Plenum Press; 1988:11–23.
14. Loveland, B. E.; Jarrott, B.; McKenzie, I. F. C. The detection of beta adrenoceptors on murine lymphocytes. Int. J. Immunopharmacol. 3:45–55; 1981.
15. Lysle, D. T.; Cunnick, J. E.; Fowler, H.; Rabin, B. S. Pavlovian conditioning of shock-induced suppression of lymphocyte reactivity: Acquisition, extinction, and preexposure effects. Life Sci. 42:2185–2194; 1988.
16. Lysle, D. T.; Cunnick, J. E.; Wu, R.; Caggiula, A. R.; Wood, P. G.; Rabin, B. S. 2-Deoxy-D-glucose modulation of T-lymphocyte reactivity: Differential effects on lymphoid compartments. Brain Behav. Immun. 2:212–221; 1988.
17. Lysle, D. T.; Lyte, M.; Fowler, H.; Rabin, B. S. Shock-induced modulation of lymphocyte reactivity: Suppression, habituation, and recovery. Life Sci. 41:1805–1814; 1987.
18. Maier, S. F.; Laudenslager, M. L. Inescapable shock, shock controllability, and mitogen stimulated lymphocyte proliferation. Brain Behav. Immun. 2:87–91; 1988.
19. Munck, A.; Guyre, P. M.; Holbrook, M. J. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5:25–44; 1984.
20. Murphy, B. E. Some studies on the protein binding of steroids and their application to the routine micro and ultra-micro measurement of various steroids in body fluids by competitive protein-binding radioassay. J. Clin. Endocrinol. Metab. 27:973–990; 1967.
21. Okimura, T.; Ogawa, M.; Yamauchi, T. Stress and immune responses III. Effect of restraint stress on delayed type hypersensitivity (DTH) response, natural killer (NK) activity and phagocytosis in mice. Jpn. J. Pharmacol. 41:229–235; 1986.
22. Pollock, R. E.; Babcock, G.; Romsdahl, M. M.; Nishioka, K. Surgical stress suppression of murine natural killer cell cytotoxicity. Cancer Res. 44:3888–3891; 1984.
23. Pollock, R. E.; Lotzová E.; Stanford, D.; Romsdahl, M. M. Effect of surgical stress on murine natural killer cell cytotoxicity. J. Immunol. 138:171–178; 1987.
24. Post, R. M. Intermittent versus continuous stimulation: Effect of the interval on the development of sensitization or tolerance. Life Sci. 26:1275–1282; 1980.
25. Rivier, C.; Vale, W. Interaction between ethanol and stress on ACTH and β-endorphin secretion. Alcohol.: Clin. Exp. Res. 12:206–210; 1988.
26. Scarpace, P. J.; Baresi, L. A.; Morley, J. E. Glucocorticoids modulate beta-adrenoceptor subtypes and adenylate cyclase in brown fat. Am. J. Physiol. 255:E153-E158; 1988.
27. Shavit, Y.; Lewis, J. W.; Terman, G. W.; Gale, R. P.; Liebeskind, J. C. Opioid peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity. Science 223:188–190; 1984.
28. Stern, L.; Kunos, G. Synergistic regulation of pulmonary beta-adrenergic receptors by glucocorticoids and interleukin-1. J. Biol. Chem. 263:15876-15879; 1988.
29. Weber, R. J.; Pert, A. The periaqueductal gray matter mediates opiate-induced immunosuppression. Science 245:188–190; 1989.