3-Aminobenzamide prevents restraint-evoked immunocompromise

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Abstract

Chronic stressors compromise immune function, which may affect disease state in rats and mice. Although the molecular mechanism(s) underlying the link between psychological stressors and physiological responses remain elusive, one putative mechanism is oxidative stress. DNA damage activates poly(ADP-ribose) polymerase (PARP), a nuclear enzyme that participates in DNA repair; if DNA damage is extensive, however, then PARP becomes cytotoxic. Because PARP-1−/− transgenic mice are resistant to chronic stress-induced immunocompromise, we tested the hypothesis that pre-restraint administration of 3-aminobenzamide (3-AB), a PARP inhibitor, would prevent restraint-evoked suppression of antibody production to the novel protein, keyhole limpet hemocyanin (KLH). Mice were physically restrained for 3 h daily for 14 consecutive days, then immunized with KLH. Daily restraint continued for an additional 21 days and anti-KLH IgG production was assessed. Mice exposed to repeated restraint reduced concentrations of anti-KLH IgG, whereas, mice treated with 3-AB (0.5, 5.0, or 20.0 mg/kg) prior to each bout of restraint displayed anti-KLH IgG concentrations similar to those of unrestrained mice. Treatment with 3-AB (0.5 and 5.0 mg/kg) during the restraint paradigm also facilitated habituation of the corticosterone response to restraint, and 3-AB (0.5 mg/kg) reduced the effect of repeated restraint on body mass. However, the immunoprotective effects of 3-AB and the endocrine and metabolic effects appear to be distinctively regulated because, unlike the endocrine and metabolic effects, the immunoprotective effects of 3-AB were independent of dose. These data suggest that PARP inhibitors may be useful to prevent compromised immune function in response to stressors.

Keywords: PARP; Immune; Stress; Restrained; KLH

1. Introduction

Psychological stressors contribute to disease onset and progression (Amick et al., 2002; Balbin et al., 1999; Chrousos, 2000a,b; Yang and Glaser, 2000) and the results of laboratory studies suggest that chronic stressors compromise immune function in rats and mice (e.g., Dhabhar and McEwen, 1999; Neigh et al., 2004a,b; Tournier et al., 2001). Oxidative stress is one possible link between the experience of psychological stress and physiological changes that result in compromised immune function. Oxidative DNA injury is suggested by an increase in 8-hydroxydeoxyguanosine (8-OH-dG), and the severity of psychological stressors is positively correlated with 8-OH-dG concentrations in leukocytes (Irie et al., 2001), suggesting that psychological stress co-occurs with oxidative DNA injury in immune cells.

Damage to DNA activates poly(ADP-ribose) polymerase (PARP), a nuclear enzyme that participates in DNA repair. The normal function of PARP requires the use of both NAD⁺ and ATP. If DNA damage is extensive, then PARP becomes cytotoxic through an excessive consumption of NAD⁺ and ATP resulting in necrotic cell death and by acting as a signal for apoptosis (Ha and Snyder, 2000; Virag and Szabo, 2002). PARP may
mediate stress-evoked immunocompromise (Drazen et al., 2001). Repeated exposure of PARP-1−/− mice to forced swim did not affect immune function; in contrast, wild-type mice experiencing the same repeated stressors displayed reduced splenocyte proliferation and reduced antibody production in response to a novel protein (Drazen et al., 2001).

Inhibitors of PARP are effective in minimizing oxidative damage following stroke (Takahashi et al., 1999), global cerebral ischemia (Plaschke et al., 2000), inflammatory diseases (Chiarugi, 2002; Mabley et al., 2001; Scott et al., 2004), and circulatory shock (McDonald et al., 2000; Veres et al., 2003). PARP inhibitors limit the action of PARP by either blockade of NAD+ binding to the catalytic domain of PARP or inhibition of the binding of PARP to DNA (Virag and Szabo, 2002). If PARP activation plays a role in stress-evoked reductions in immune function, then administration of a PARP inhibitor should block compromise of immune function in response to chronic stressors. In the present study, we tested the hypothesis that administration of the PARP inhibitor 3-aminobenzamide (3-AB) prior to each bout of restraint would prevent the restraint-evoked reduction of antibody production in response to a novel antigen.

2. Methods

2.1. Animals

The Institutional Laboratory Animal Care and Use Committee of Ohio State University approved all animal procedures. All studies were conducted in accordance with the guidelines set forth by the National Institutes of Health and the US Department of Agriculture. Adult male C57BL/6 mice (12 weeks) were housed individually in polycarbonate cages (28 × 17 × 12 cm) from the onset of the study in rooms programmed to be illuminated for 14h/day (LD14:10, lights illuminated at 01:00 h Eastern Standard Time (EST)) and maintained at temperatures of 20 ± 4°C and relative humidity of 50 ± 5%. Food and filtered tap water were available ad libitum throughout the study. Mice were randomly assigned to one of eight experimental groups: (1) saline injection (n = 7), (2) 0.5 mg/kg 3-AB (n = 8), (3) 5.0 mg/kg 3-AB (n = 8), (4) 20.0 mg/kg 3-AB (n = 8), (5) restraint + saline (n = 7), (6) restraint + 0.5 mg/kg 3-AB (n = 8), (7) restraint + 5.0 mg/kg 3-AB (n = 8), and (8) restraint + 20.0 mg/kg 3-AB (n = 8).

2.2. Restraint

Mice were placed in ventilated clear polypropylene restrainers (50 ml conical tubes measuring 9.7 cm in length and internal diameter of 2.8 cm) for 3h/day for 5 weeks; breathing was monitored for all mice to make certain animals were not compressed. Restraint onset was randomized but always occurred between 07:00 and 09:00 h EST.

2.3. 3-Aminobenzamide

3-AB (Sigma, St. Louis, MO) was dissolved in sterile 0.9% sodium chloride and administered by intraperitoneal (i.p.) injection 30 min prior to the onset of restraint. The drug was prepared such that mice received a 0.1 ml injection regardless of the dose. Sodium chloride (0.9%) was administered as the control solution and three doses of 3-AB were tested (0.5, 5.0, and 20.0 mg/kg). These doses were chosen because they are within the range of therapeutic doses previously demonstrated (Bowes and Thiemermann, 1998; Couturier et al., 2003; Ozdulger et al., 2002).

2.4. Anti-KLH IgG

To determine humoral immune response, mice were injected with the antigen, keyhole limpet hemocyanin (KLH; 150 μg suspended in 0.1 ml sterile 0.9% sodium chloride), to which they were previously naïve, on day 14 of the experiment. KLH evokes an acute immune response, but does not replicate or cause long-term fever or inflammation at the dose used (Dixon et al., 1966). IgG specific for KLH was measured on days 3, 7, 14, and 21 post-immunization using a specific enzyme-linked immunosorbent assay (ELISA). Diluted serum samples (1:50) were added to antigen coated plates that were previously blocked with 0.5% milk in PBS to reduce nonspecific binding. After incubation, secondary antibody (AP-conjugated anti-mouse IgM, 1:1000) was added, and after further incubation, the enzyme substrate p-nitrophenyl phosphate was added. The OD of each well was determined using a Bio-Rad plate reader (405 nm). Mean OD for each sample was expressed as a percent plate positive control for statistical analyses.

2.5. Corticosterone RIA procedures

All blood samples were obtained from the retro-orbital sinus under light isoflurane anesthesia and serum corticosterone was determined by radioimmunoassay (RIA). We conducted the RIA following the guidelines in the ICN (Costa Mesa, CA) 125I double-antibody kit instructions. The RIA is highly specific, cross-reacting at less than 1% with other hormones and a detection limit of 5 ng/ml. All samples were assessed in one assay and the intra-assay coefficients of variation were <10%. Corticosterone concentrations were measured from blood collected 2 days prior to experiment onset, and on days 1, 21, 28, 35, and 36 to assess the effect of each treatment. On days −2 and 36, blood samples were collected within 2 min of disturbing the
cage. This period of time is sufficiently short to allow for collection of basal corticosterone (Riley, 1981). On days 1, 21, 28, and 35, blood samples were collected 3.5 h after injection and 5 min post-restraint.

2.6. Data analyses and statistics

Two-way repeated measures ANOVAs were used to assess differences of treatment and time for humoral immune function and corticosterone concentrations. The initial body mass, terminal body mass and the percent change in body mass, were assessed with one-way ANOVAs. Tukey pairwise multiple comparisons and t tests were used to further distinguish among groups. Mean differences were considered statistically significant if \( p < .05 \) unless the assumptions of normality or equal variance were violated. If these assumptions were violated, then we adjusted to \( p < .025 \) for initial analyses and \( p < .01 \) for post hoc analyses to correct for the increased likelihood of Type I error (Keppel, 1991).

3. Results

3.1. 3-AB prevents restraint-induced suppression of anti-KLH IgG concentrations

Repeated restraint suppressed anti-KLH IgG concentrations as compared to mice that were exposed to repeated saline injections (Treatment: \( F(1,4) = 5.6, p < .025 \); Time: \( F(4,60) = 13.6, p < .025 \); Treatment × Time: \( F(4,69) = 1.6, p > .025 \); Fig. 1). Treatment with 3-AB (0.5, 5.0, or 20.0 mg/kg) prevented the restraint-induced suppression of anti-KLH IgG concentrations such that the concentrations of anti-KLH IgG in these groups did not differ from saline injected mice (Treatment: \( F(3,12) = 0.09, p > .025 \); Time: \( F(4,33) = 29.9, p < .025 \); Treatment × Time: \( F(12,152) = 0.2, p > .025 \)). Treatment with 3-AB (0.5, 5.0, or 20.0 mg/kg), in the absence of restraint, did not alter anti-KLH IgG concentrations as compared to saline injected mice (Treatment: \( F(3,12) = 1.3, p > .025 \); Time: \( F(4,135) = 28.1, p < .025 \); Treatment × Time: \( F(12,135) = 0.5, p > .025 \)). Mice exposed to saline injections, 3-AB, or 3-AB and restraint had elevated concentrations of anti-KLH IgG, as compared to their respective baseline concentrations, 7, 14, and 21 days after exposure to KLH (\( p < .01 \)). Mice exposed to repeated restraint had elevated concentrations of anti-KLH IgG, as compared to their baseline concentrations, 14 and 21 days after exposure to KLH (\( p < .01 \)).

3.2. 3-AB facilitates habituation of the corticosterone response to restraint

Exposure to repeated injections (saline, 0.5, 5.0, and 20.0 mg/kg 3-AB) and exposure to repeated injections and restraint differentially altered corticosterone concentrations (Treatment: \( F(7,35) = 49.5, p < .025 \); Time: \( F(5,324) = 66.1, p < .025 \); Treatment × Time: \( F(35,324) = 7.4, p < .025 \)). Baseline corticosterone concentrations were similar regardless of group assignment both prior to the onset of the experiment and 24 h following the final treatment (\( p > .01 \)). There were no differences in corticosterone concentrations following injection of either saline or 3-AB (0.5, 5.0, or 20.0 mg/kg) among the groups or with respect to their respective baselines (\( p > .01 \)). All groups exposed to restraint increased concentrations of corticosterone following the first exposure to restraint as compared to baseline concentrations (\( p < .01 \); Fig. 2) and there were no differences in concentrations among the
groups \((p > .01)\). Following 21 days of exposure to restraint, all groups had lower restraint-evoked corticosterone concentrations than the concentrations observed after the first exposure \((p < .01)\). The mice given 3-AB \((0.5 \text{ mg/kg})\) prior to restraint had corticosterone concentrations similar to their baseline values on day 21 of restraint \((p > .01)\). After 35 days of restraint, mice given 3-AB \((5.0 \text{ mg/kg})\) prior to restraint had corticosterone concentrations similar to their baseline values \((p > .01)\) and lower than mice treated with saline prior to restraint \((p < .01)\). Mice treated with 3-AB \((0.5 \text{ or } 20.0 \text{ mg/kg})\) prior to restraint had corticosterone concentrations higher than those treated with 3-AB \((5.0 \text{ mg/kg})\) on day 35 of restraint \((p < .01)\).

3.3. 3-AB prevents restraint-induced loss of body mass

Body mass did not differ among the groups at the start of the experiment \((F(7,54)=0.8, p > .05)\), but terminal body mass differed based on treatment \((F(7,54)=3.4, p < .05)\). The percent change in body mass over the course of the experiment was lower for the group exposed to restraint \((F(7,54)=2.5, p < .05; \text{Fig. 3})\). Post hoc comparison demonstrated that mice exposed to repeated restraint gained less body mass than either mice injected with saline but not restrained \((t(12)=2.8, p < .05)\) or mice treated with 3-AB \((0.5 \text{ mg/kg})\) prior to restraint \((t(13)=3.1, p < .05)\).

4. Discussion

Activation of PARP may be a critical step in the process of reduced immune function in response to exposure to chronic stressors. The data presented in this paper demonstrate that treatment with 3-AB, a pharmacological inhibitor of PARP, prevented restraint-evoked immunosuppression. Mice treated with 3-AB prior to each exposure to restraint had similar concentrations of anti-KLH IgG as mice exposed to a daily injection without restraint. Preserved immune function in restrained mice following injection of 3-AB contrasted with the reduced concentrations of anti-KLH IgG for mice given saline prior to each exposure to restraint (Fig. 1). Although 3-AB has anti-oxidant properties (reviewed in Virag and Szabo, 2002), the function of 3-AB as a PARP inhibitor is supported by a previous examination of stress effects on immune function in PARP-1\(^{-/-}\) knockout mice. PARP-1\(^{-/-}\) knockout mice do not reduce splenocyte proliferation or impair antibody production following repeated stressors, as compared to wild-type mice (Drazen et al., 2001). The converging evidence provided by examination of stress effects on immune function in both PARP-1\(^{-/-}\) knockout mice (Drazen et al., 2001) and wild-type mice treated with 3-AB (Fig. 1) suggests that activation of PARP during chronic stress may be a mechanism by which psychological stressors inhibit some features of immune function.

Treatment with 3-AB hastened habituation of the corticosterone response to restraint in those mice.
treated with either 0.5 or 5.0 mg/kg of 3-AB (Fig. 2). Although 3-AB treatment (0.5 or 5.0 mg/kg) altered dynamics of the corticosterone response to restraint, it seems unlikely that this change in corticosterone mediated the immunoprotective effects of 3-AB because mice treated with the 20.0 mg/kg 3-AB do not demonstrate an altered corticosterone response to repeated restraint, but in common with mice treated with 0.5 or 5.0 mg/kg of 3-AB, immune function was not impaired by restraint. PARP-1−/− mice and wild-type mice have similar corticosterone concentrations in response to forced swim, a common laboratory stresor, and PARP-1−/− mice are protected from dexamethasone-induced immunosuppression (Drazen et al., 2001). Our data confirm and extend these previous results, and suggest that PARP mediates immunosuppression downstream of glucocorticoid secretion. Glucocorticoids play a role in immune cell death (e.g., Bauer et al., 2001) and elevated glucocorticoid secretion may stimulate PARP activity. Excessive activation of PARP initiates a biochemical cascade that results in cell death (Virag and Szabo, 2002) and this mechanism may contribute to glucocorticoid-induced cell death.

3-AB prevented the effects of restraint on body mass, suggesting a metabolic function of the drug. Mice treated with 3-AB (0.5 mg/kg) prior to each exposure to restraint gained more body mass than those mice given saline prior to restraint (Fig. 3). Although metabolic changes can affect immune function (e.g. Austin et al., 1980; Dreau et al., 1998; Lord et al., 1998), the influences of 3-AB on the immune system and body mass may not be directly related. Immune function is preserved following all doses of 3-AB (0.5, 5.0, and 20.0 mg/kg), yet only the 0.5 mg/kg dose of 3-AB prevents the restraint-induced change in body mass. PARP inhibitors, such as 3-AB, may alter metabolism, stimulate food intake (not measured in this study), or both. Insulin is reduced during repeated exposure to restraint (Neigh et al., 2004b) and PARP inhibitors can influence insulin secretion. PARP inhibitors are protective against hypoglycemia-induced cell death (Suh et al., 2003) and maintain insulin secretion by protecting damaged β-cell islets and stimulating regeneration of β-cell islets in the pancreas (Takasawa and Okamoto, 2002). Therefore, PARP inhibitors may alter body mass through changes in insulin secretion.

Administration of 3-AB during exposure to repeated restraint preserves immune function, facilitates the habituation of the corticosterone response to restraint, and inhibits restraint-induced loss of body mass. Although the mechanism of action is not yet known, PARP inhibitors appear to intervene downstream of glucocorticoid secretion. These data add to previous reports that use of pharmacological PARP inhibitors may be useful tools for the prevention of physiological disorders.

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References


