Am J Physiol Regul Integr Comp Physiol 285: R873–R879, 2003;
10.1152/ajpregu.00209.2003.

Photoperiod modulates the effects of norepinephrine on lymphocyte proliferation in Siberian hamsters

Gregory E. Demas,[1] Timothy J. Bartness,[2] Randy J. Nelson,[3] and Deborah L. Drazen[4]

[1]*Department of Biology and Program in Neural Science, Indiana University, Bloomington, Indiana 47405;* [2]*Department of Biology and Center for Behavioral Neuroscience, Georgia State University, Atlanta, Georgia 30303;* [3]*Departments of Psychology and Neuroscience, Ohio State University, Columbus, 43210; and* [4]*Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267*

Submitted 18 April 2003; accepted in final form 15 June 2003

Demas, Gregory E., Timothy J. Bartness, Randy J. Nelson, and Deborah L. Drazen. Photoperiod modulates the effects of norepinephrine on lymphocyte proliferation in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* 285: R873–R879, 2003; 10.1152/ajpregu.00209.2003.—Siberian hamsters (*Phodopus sungorus*) rely on photoperiod to coordinate seasonally appropriate changes in physiology, including immune function. Immunity is regulated, in part, by the sympathetic nervous system (SNS), although the precise role of the SNS in regulating photoperiodic changes in immunity remains unspecified. The goal of the present study was to examine the contributions of norepinephrine (NE), the predominant neurotransmitter of the SNS, to photoperiodic changes in lymphocyte proliferation. In *experiment 1*, animals were maintained in long [16:8-h light-dark cycle (16:8 LD)] or short days (8:16 LD) for 10 wk, and splenic NE content was determined. In *experiment 2*, in vitro splenocyte proliferation in response to mitogenic stimulation (concanavalin A) was assessed in spleen cell suspensions taken from long- or short-day hamsters in which varying concentrations of NE were added to the cultures. In *experiment 3*, splenocyte proliferation was examined in the presence of NE and selective α- and β-noradrenergic receptor antagonists (phenoxybenzamine and propranolol, respectively) in vitro. Short-day animals had increased splenic NE content compared with long-day animals. Long-day animals had higher proliferation compared with short-day animals independent of NE. NE (1 μM) further suppressed splenocyte proliferation in short but not long days. Last, NE-induced suppression of proliferation in short-day hamsters was blocked by propranolol but not phenoxybenzamine. The present results suggest that NE plays a role in photoperiodic changes in lymphocyte proliferation. Additionally, the data suggest that the effects of NE on proliferation are specific to activation of β-adrenergic receptors located on splenic tissue. Collectively, these results provide further support that photoperiodic changes in immunity are influenced by changes in SNS activity.

immune; seasonal; stress; sympathetic nervous system; catecholamines

MANY NONTROPICAL MAMMALIAN species undergo seasonal changes in physiology and behavior (8, 22). In addition to the traditionally studied seasonal changes in reproduction and energy balance (2, 34), there are pronounced fluctuations in immunity and disease susceptibility (reviewed in Refs. 37, 38). For example, field studies of seasonal changes in immunity typically report reduced immune function and increased disease susceptibility during winter compared with spring and summer (24, 29, 30, 38). In addition, photoperiodic changes in immune function have been documented in several rodent species, including deer mice (*Peromyscus maniculatus*) (10, 15) and prairie voles (*Microtus ochrogaster*) (39), as well as Syrian (*Mesocricetus auratus*) (7) and Siberian (*Phodopus sungorus*) (18, 48) hamsters. In some species (e.g., Siberian hamsters, prairie voles), specific immune responses (e.g., antibody production) are suppressed in short days, in accordance with the majority of field studies. In contrast, individuals of other species (e.g., deer mice, Syrian hamsters) display enhanced immune function in short winterlike day lengths compared with long summerlike days. Although the mechanisms for these differences remain unknown, they likely reflect differences in evolutionary pressures that have shaped physiological responses in these species. It has been suggested that photoperiodic changes in immune function may represent adaptive functional responses to seasonal changes in the energetic budgets of small rodents. In other words, all physiological responses are energetically costly; animals may have evolved to reduce immune function at certain times of the year when energy intake is low and energy expenditure is high (29, 37, 38). The precise physiological mechanisms mediating photoperiodic changes in immune function, however, have not been fully characterized.

It is well established that the sympathoadrenal system (i.e., direct sympathetic innervation of lymphoid tissue, as well as secretion of adrenal catecholamines) can have profound effects on immunity. Because of the rather extensive literature demonstrating suppression of immunity by chronic adrenal glucocorticoids (e.g.,

Address for reprint requests and other correspondence: G. E. Demas, Dept. of Biology, Indiana Univ., Bloomington, IN 47405 (E-mail: gdemas@bio.indiana.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

corticosterone, cortisol) (reviewed in Refs. 16, 41), sympathoadrenal contributions to immune regulation have been largely overlooked. Recent evidence, however, suggests that a wide range of environmental and intrinsic stressors can activate the sympathetic nervous system (SNS) and suppress immune function (reviewed in Refs. 32, 43). Anatomic studies also have established the direct innervation of lymphoid tissue by the SNS in a variety of species (4, 45). Norepinephrine (NE), the primary postganglionic sympathetic neurotransmitter, appears to be the predominant neurotransmitter phenotype of these neurons; NE is released from nerve terminals immediately adjacent to lymphocytes, as well as from more distant sites acting in a paracrine fashion (reviewed in Ref. 32). Moreover, NE released from these nerves can have profound effects on both humoral and cell-mediated immunity (19, 20, 33). The actions of NE on immunity appear to be direct; adrenoreceptors (AR) have been localized on lymphoid tissue (32), and NE can alter immune function in vitro (26, 33, 40).

NE exerts its effects on target cells by acting on two principal adrenoreceptor subtypes, α- and β-AR. Nearly all lymphoid cells express β-AR, although evidence suggests that β-AR differ in density depending on the specific type of lymphoid cell (43). Circulating lymphocytes and natural killer (NK) cells also express β-AR, although the number of β-AR varies during the lifespan of the cell. Other immune cells, such as B cells, also possess low-affinity β-AR. The existence of specific subtypes of α-AR on immune cells, however, remains controversial. Some studies suggest that both α_1- and α_2-AR are expressed on immune cells (19), and there are reports of α-AR expressed on human lymphocytes. In addition, activated rodent macrophages express α_2-AR (33); however, α-AR have not been identified in rodent lymphocytes.

The broad goal of the present study was to test the hypothesis that the SNS, via direct innervation of lymphoid tissue and concomitant release of NE, plays a role in photoperiodic changes in immune function in Siberian hamsters. Recently, direct SNS innervation of the spleen has been demonstrated in Siberian hamsters using the retrograde transneuronal tract tracer pseudorabies virus (PRV) (17). Specifically, PRV injected into the spleens of Siberian hamsters revealed infected neurons in areas traditionally implicated in SNS regulation (e.g., A5 and C1 cell groups, locus ceruleus). Labeled neurons were also identified in the paraventricular (PVN) and suprachiasmatic nuclei (SCN) of the hypothalamus (17), brain regions critical for the transduction of the photoperiodic signal (22). More recently, our laboratory has demonstrated that the SNS plays an important functional role in regulating photoperiodic changes in immune function in Siberian hamsters. Specifically, short day-housed hamsters receiving surgical denervation of the spleen displayed reduced humoral immunity compared with sham-operated hamsters; splenic denervation, however, had no effect on immunity of long-day hamsters (13). These results suggest that the SNS is associated with photoperiodic changes in immune function in Siberian hamsters. The specific aims of the present study were *1*) to examine the effects of photoperiod on NE content within the spleen, *2*) to test the effects of exogenous NE on lymphocyte proliferation, and *3*) to identify the AR subtypes involved in mediating these potential effects.

MATERIALS AND METHODS

Animals and housing conditions. Adult (>60 days of age) male Siberian hamsters (*Phodopus sungorus*) were obtained from breeding colonies at Johns Hopkins University (*experiments 1* and *2*) and Indiana University (*experiment 3*). The research with animals conforms to federal National Institutes of Health and United States Department of Agriculture regulations and was conducted with approval of our local Institutional Animal Care and Use Committees. Hamsters were weaned at 21 days of age and housed with same-sex siblings. Two weeks before the initiation of the experiments, animals were housed individually in polypropylene cages (27.8 × 7.5 × 13.0 cm) in colony rooms with a 24-h [16:8-h light-dark (LD 16:8)] cycle (lights on 0300 EST). Temperature was kept constant at 20 ± 2°C, and relative humidity was maintained at 50 ± 5%. Food (Purina Rat Chow) and tap water were available ad libitum throughout the experiment.

Experiment 1: effects of photoperiod in splenic NE content. The goal of *experiment 1* was to determine the effects of photoperiod on NE content within the spleens of Siberian hamsters. Hamsters (n = 40) were selected randomly and assigned to either long (LD 16:8) (n = 20) or short days (LD 8:16) (n = 20). Animals were maintained in their respective photoperiods for 10 wk. Animals were then brought one at a time into the surgery room and killed by cervical dislocation; paired testes and spleens were removed, cleaned of connective tissue, and weighed at necropsy. Spleens were then snap-frozen in liquid nitrogen to preserve catecholamine content and immediately stored at −80°C freezer until assayed. All necropsies occurred between 1000 and 1100 EST to control for circadian rhythms in lymphocyte proliferation.

Experiment 2: effects of in vitro NE on splenocyte proliferation. The goal of *experiment 2* was to test the effects of exogenous NE on lymphocyte proliferation in response to mitogenic stimulation. Hamsters (n = 25) were selected randomly and assigned to either long (n = 11) or short days (n = 14) for 10 wk and were then killed by cervical dislocation between 1000 and 1100 EST. Paired testes were removed, cleaned of connective tissue, and weighed at necropsy to determine photoperiodic responsiveness. Spleens were also removed under aseptic conditions, weighed, and immediately suspended in culture medium (RPMI-1640/HEPES).

Experiment 3: effect of in vitro α- and β-adrenergic antagonists on splenocyte proliferation. The goal of *experiment 3* was to determine which AR subtypes are involved in NE-induced reductions in lymphocyte proliferation in response to mitogenic stimulation. Hamsters (n = 26) were selected randomly and assigned to either long (n = 10) or short days (n = 16) for 10 wk and were then killed by cervical dislocation between 1000 and 1100 EST. Paired testes were removed, cleaned of connective tissue, and weighed at necropsy. Spleens were also removed under aseptic conditions, weighed, and immediately suspended in culture medium (RPMI-1640/HEPES).

HPLC determination of catecholamine content. Splenic NE content was determined in *experiment 1* by measuring NE content in the spleen by reverse-phase high-pressure liquid chromatography with electrochemical detection (HPLC-EC) according to the method of Ref. 12 after Ref. 35. Briefly,

tissue was thawed, weighed, and carefully minced. A 250-mg sample was added to a microcentrifuge tube containing 790 μl of 0.2 M perchloric acid. Ten microliters of dihydroxybenzoacetic acid (DHBA; 2 μg/ml) was added to each sample and served as an internal standard. Tissue was further minced and then sonicated for 5 min on ice (5× for each sample). Catecholamines were extracted from the remaining infranatant by adding alumina (200 mg/sample), vortexing for 30 s, followed by 1 ml of 0.5 M Tris (pH = 8.6). Last, 200 μl of perchloric acid was added to elute the catecholamines. Tris buffer was aspirated using a micropipette, and the extracted samples were assayed using an ESA HPLC system with electrochemical detection (guard cell: +35 mV; cell 1: +10 mV; cell 2: −30 mV; Chelmsford, MA). The mobile phase was Cat-A-Phase II purchased from a commercial supplier (ESA). Standard solutions (50, 33, 16.5 ng/ml) were prepared from commercially supplied standard kit (ESA) and were run at the beginning, in the middle, and at the end of the sets of unknowns. Assay results were analyzed offline and expressed as nanograms per gram tissue. The sensitivity of this assay was 15 pg.

Lymphocyte proliferation. Lymphocyte proliferation in response to the T-cell mitogen, concanavalin A (Con A), was determined using a colorimetric assay based on the tetrazolium salt 3-(4,5-demethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (11). Splenocytes were separated from tissue by compressing the whole spleen between sterile frosted glass slides; separated cells were suspended in 4 ml of culture medium [RPMI-1640/HEPES supplemented with 1% penicillin (5,000 U/ml)/streptomycin (5,000 μg/ml), 1% L-glutamine (2 mM/ml), 0.1% 2-mercaptoethanol (5×10^{-2} M/ml), and 10% heat-inactivated fetal bovine serum]. Splenocyte counts and viability were determined with a hemacytometer and trypan blue exclusion. Viable cells (which exceeded 95%) were adjusted to 2×10^6 cells/ml by dilution with culture medium. In a pilot assay, Con A (Sigma Chemical, St. Louis, MO) was diluted with culture medium to concentrations of 0.6, 1.25, 2.5, 5, 10, 20 and 40 μg/ml; 50 μl of each mitogen concentration was added to the wells of the plate containing the spleen cell suspensions to yield a final volume of 150 μl/well (each in duplicate). These concentrations yielded a typical dose-response curve with proliferation absorbance values of 0.37 ± 0.02, 0.39 ± 0.26, 0.46 ± 0.03, 0.53 ± 0.07, 0.80 ± 0.20, 1.10 ± 0.12, 1.31 ± 0.17, and 0.53 ± 0.06, respectively. Based on these values, 20 μg/ml concentration of Con A was chosen as the "optimal" dose because it resulted in maximal mitogen-induced proliferation (as measured by absorbance); this Con A concentration was used in subsequent proliferation assays in *experiments 2* and *3*.

In *experiment 2*, 50-μl aliquots of each cell suspension (i.e., 100,000 cells) were added to each of three wells of sterile flat-bottom 96-well culture plates. One well then received 50 μl of 1.0 μM NE, another well received 50 μl of 10.0 nM NE, and the last well received 50 μl of culture medium. Con A was diluted with culture medium to a concentration of 20 μg/ml; 50 μl of each of the diluted mitogens was added to the wells of the plate containing the spleen cell suspensions to yield a final volume of 150 μl/well (each in duplicate).

In *experiment 3*, 50-μl aliquots of each cell suspension were added to each of four wells of sterile flat-bottom 96-well culture plates. One well received 50 μl of NE (1 μM) dissolved in culture media, another well received NE (1 μM) + propranolol (5 μM) in culture media, the third well received NE (1 μM) + phenoxybenzamine (5 μM), and the last well received culture media alone (no NE). In a previous pilot experiment, we demonstrated that neither propranolol nor phenoxybenzamine administered alone (i.e., without NE) has any effect on Con A-induced splenocyte proliferation in long- or short-day hamsters (Demas, unpublished data). As in *experiment 2*, an optimal dose of Con A (20 μg/ml) was added to all spleen cell suspensions.

The plates from both *experiments 2* and *3* were incubated at 37°C with 5% CO_2 for 48 h before addition of 20 μl of MTS/phenazine methosulfate (PMS) solution [Promega; 0.92 mg/ml of PMS in sterile Dulbecco's phosphate-buffered saline] per well. Plates were then incubated at 37°C with 5% CO_2 for an additional 4 h. The optical density (OD) of each well was determined with a microplate reader (Bio-Rad: model 3550) equipped with a 490-nm wavelength filter. Mean OD values for each set of duplicates were used in subsequent statistical analyses. In *experiment 2*, dose-response curves were constructed using group means of the mean OD values at each mitogen concentration and unstimulated cultures.

Statistical analyses. Differences in testes masses in all three experiments and between splenic NE content in *experiment 1* were assessed by separate independent Student's *t*-tests. Differences in splenocyte proliferation in *experiments 2* and *3* were assessed via a two-way (photoperiod × drug) between-groups ANOVA. Post hoc comparisons between pairwise means were conducted using Tukey's honestly significant difference tests when the overall ANOVAs were significant. All statistical tests were conducted using SigmaStat Statistical software (San Rafael, CA). In all cases, differences between group means were considered statistically significant if $P < 0.05$.

RESULTS

Experiment 1. Short day-housed hamsters had significantly smaller paired testes masses compared with long-day animals ($P < 0.05$; Fig. 1*A*), attesting to their photoresponsiveness. Short day-housed hamsters had significantly higher splenic NE content compared with long-day animals ($P < 0.05$; Fig. 1*B*).

Experiment 2. As in *experiment 1*, short day-housed hamsters had significantly smaller paired testes masses compared with long-day animals ($P < 0.05$; data not shown). Short day-housed hamsters had significantly reduced splenocyte proliferation in response to Con A compared with long-day hamsters ($P < 0.05$; Fig. 2). In addition, in vitro NE (1 μM) but not NE (10 nM) significantly reduced splenocyte proliferation in short-day hamsters ($P < 0.05$); neither concentration of NE had an effect on splenocyte proliferation in long-day hamsters ($P > 0.05$; Fig. 2). There were no differences in splenic masses between long- and short-day hamsters ($P > 0.05$).

Experiment 3. As in *experiment 1*, short day-housed hamsters had significantly smaller paired testes masses compared with long-day animals ($P < 0.05$; data not shown). Short day-housed hamsters had significantly reduced splenocyte proliferation in response to Con A compared with long-day hamsters, and in vitro NE significantly reduced splenocyte proliferation in short-day hamsters ($P < 0.05$; Fig. 3). In addition, NE-induced suppression of proliferation in short-day hamsters was blocked by the presence of in vitro propranolol, but not phenoxybenzamine ($P < 0.05$; Fig. 3). There were no differences in splenic mass between long- and short-day hamsters.

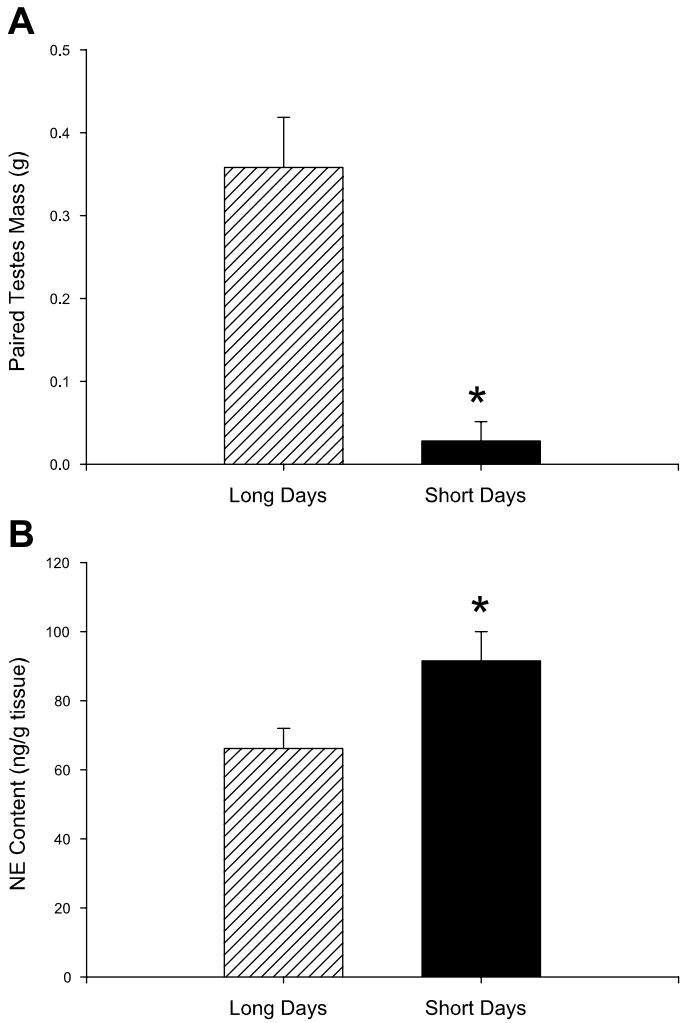

Fig. 1. Mean (±SE) paired testes mass (g; *A*) and splenic norepinephrine (NE) content (*B*) of animals housed in long [16:8-h light-dark cycle (LD 16:8)] or short (LD 8:16) days. *Significant differences between long- and short-day NE content ($P < 0.05$).

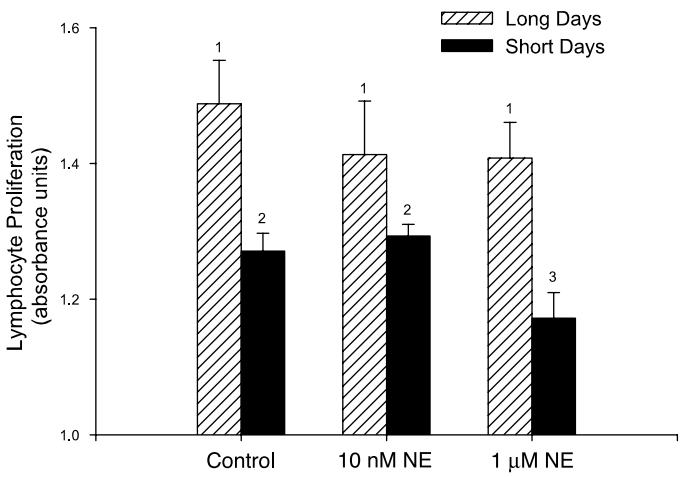

Fig. 2. Mean (±SE) splenocyte proliferation of animals housed in long (LD 16:8) or short (LD 8:16) days and with no NE (Control), 10.0 nM NE, or 1 μM NE added in vitro. Columns sharing the same number are statistically equivalent. Columns with different numbers are statistically different ($P < 0.05$).

DISCUSSION

The present data demonstrate that short-day hamsters have increased splenic NE content compared with long day-housed animals. In addition, the sympathetic neurotransmitter NE can reduce splenocyte proliferation in vitro in Siberian hamsters, and this effect is photoperiod dependent. Specifically, NE suppressed lymphocyte proliferation to Con A in short but not in long days. Furthermore, the present results support the hypothesis that the effects of NE on proliferation are mediated through β-AR; pharmacological blockade of β-AR, but not α-AR, attenuated NE-induced suppression of splenocyte proliferation. Interestingly, short days also increased splenocyte proliferation in the absence of NE in culture, suggesting that photoperiodic changes in immune cell proliferation are not completely regulated by changes in SNS activity. Collectively, the results of the present study are consistent with the idea that there are photoperiodic changes in immunity and suggest that changes in SNS activity (and subsequent NE release) may coordinate, at least in part, photoperiodic changes in lymphocyte proliferation. Furthermore, these effects appear to be mediated directly through interactions with β-AR within lymphoid tissue. These results confirm and extend the results of previous research suggesting an important role for the SNS, and specifically β-AR, in the regulation of cell-mediated immunity (23, 43). The presence of a photoperiodic effect on splenocyte proliferation independent of NE, however, suggests that NE is not absolutely required. Thus, although NE, likely acting through β-AR directly on splenocytes, can modulate photoperiodic changes in lymphocyte proliferation, factors other than the SNS (e.g., melatonin, steroid hormones) may also play an important role in mediating photoperiodic changes in lymphocyte proliferation. On-

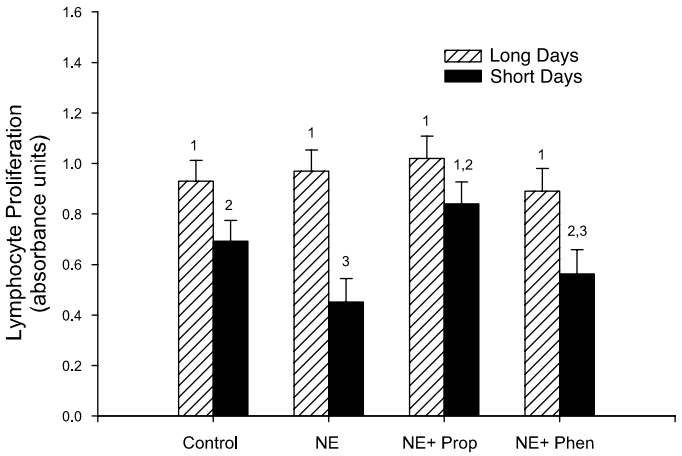

Fig. 3. Mean (±SE) splenocyte proliferation in animals housed in long (LD 16:8) or short (LD 8:16) days and with combinations of no NE (Control), 1 μM NE, or NE + 5 μM propranolol (NE + Prop) or 5 μM phenoxybenzamine (NE + Phen) added in vitro. Columns sharing at least one same number are statistically equivalent. Columns with different numbers are statistically different ($P < 0.05$).

going studies are aimed at elucidating these specific factors.

An increasing amount of evidence supports an important role of the SNS in the regulation of immunity (20, 32, 33). For example, an inhibitory role of the SNS in the regulation of immune function has been suggested in several species after denervation of a variety of lymphoid tissue (32). Specifically, global chemical denervation of the peripheral nervous system of adult animals via systemic injections of 6-hydroxydopamine (6-OHDA) enhances antibody responses to T-independent antigens but has no effect on T-independent antibody production (36). In contrast, it has also been reported that 6-OHDA-induced denervation reduces antibody responses to T-dependent antigens in adult rats (21, 28), whereas surgical denervation of the splenic nerve enhances antibody production in neonatal animals (6, 47). More recently, it has been demonstrated that chemical denervation results in a modest increase in anti-KLH antibody production in young rats, but a marked increase in antibodies in older animals (4). These results suggest that the effects of the SNS on immune function depend on several factors, including the age of the animals and the type of denervation, as well as the type of immune response measured.

The SNS is also involved in lymphocyte trafficking and circulation, with different lymphocyte populations having differing sensitivities to catecholamines (19). For example, NK cells and granulocyte circulation are both increased by catecholamines, whereas B and T cell numbers remain relatively unaffected (5, 44). In addition, 6-OHDA-induced depletion of NE increases mitogen-induced proliferation of T, but not B cells (31). A previous report, however, has demonstrated reduced Con A-induced splenocyte proliferation after NE depletion via 6-OHDA in vivo (33). Sympathectomy can exert differential effects on mitogen-induced splenocyte proliferation, with reduced proliferation in sympathectomized animals in the presence of low doses of Con A, but increased proliferation at high doses (1). Furthermore, the effects of sympathectomy on mitogen-induced splenocyte proliferation appear to be strain dependent (31); thus differences between species are likely as well. Last, as discussed above, systemic 6-OHDA results in a global SNS depletion of NE; thus it is unclear whether the decreased proliferation after 6-OHDA-induced sympathectomy is due to a direct effect of NE removal on splenocytes or, rather, an indirect effect on other immunological factors (e.g., cytokines).

The concentrations of NE used in the present study were chosen because these doses have been shown previously to result in reliable inhibition of cellular proliferation (25). The use of a high physiological dose allowed us to be confident that we would not exclude potentially subtle effects of NE on proliferation, while at the same time it enabled us to assess a meaningful, biologically relevant physiological response. Given that the effects of NE on lymphocyte proliferation in short-day hamsters occurred at high but not low levels of NE, however, it may be suggested that this effect is an artifact of potentially pharmacological levels of the neurotransmitter. Although we cannot totally rule out this possibility, we believe it is unlikely because the relatively high effective doses of NE used in these experiments are within the range of NE concentrations typically experienced within the local environment surrounding peripheral tissue (25). Regardless of whether the NE concentrations used in these studies are physiological or pharmacological, however, the results of the present demonstrate that splenocytes from short-day hamsters are more responsive to NE in vitro than long-day splenocytes.

Consistent with previous studies, the results of the present study suggest that the reduction in splenocyte proliferation in response to NE in vitro is a result of β-AR activity. For example, stimulation of β-AR can inhibit both mitogen- or anti-CD3 antibody-induced T cell proliferation (9, 23). Several subtypes of β-AR have now been identified, including β_1-, β_2-, and β_3-AR subtypes (19). Based on radioligand binding studies, mature lymphocytes appear to express predominantly β_2-AR (27), and this receptor subtype plays a primary functional role in regulating immune responses (reviewed in Ref. 42). For example, nonspecific β-AR agonists and selective β_2- but not β_1-agonists reduce interleukin-2 and γ-interferon production, as well as mitogen-induced lymphocyte proliferation (42). Although the present results suggest a role of β-AR in regulating NE-induced suppression of lymphocyte proliferation, the use of the nonspecific β-AR antagonist propranolol in the present study prevents us from determining the specific receptor subtype involved. Studies remain to be done that utilize subtype-specific antagonists and agonists and that will allow us to address these questions.

The results of the present study suggest that the SNS, and specifically NE, plays an important role in photoperiodic changes in lymphocyte proliferation. Furthermore, these results demonstrate that short days increase NE content within the spleen compared with long days. These data suggest that maintenance in short winterlike days increases SNS outflow to peripheral lymphoid tissues such as the spleen, supporting previous findings of short day-induced changes in SNS outflow to peripheral tissues in Siberian (49) and Syrian hamsters (46). The latter finding is particularly interesting in that SNS activity is decreased in short compared with long days in Syrian hamsters, a finding consistent with increased immune function reported in short days for this species. Thus it is possible that the differential effects of short days on SNS outflow may underlie the differences in photoperiodic effects on immunity reported for these hamster species, although this hypothesis requires testing. In the present study, increased SNS activity and concomitant increases in NE release in short days appear to act directly on β-AR on splenocytes to modulate lymphocyte proliferation. These data provide novel and important insights into the neuroendocrine mechanisms underlying environ-

mentally induced fluctuations in lymphocyte proliferation and likely immune function.

We acknowledge M. Dumler, A. Durazzo, K. Nies, and K. Proud for expert animal care.

DISCLOSURES

This research was supported by National Institutes of Health (NIH) Awards NS-10596 to G. E. Demas, DK-35254 to T. J. Bartness, MH-57535 to R. J. Nelson, as well as National Science Foundation Grant 00–0854 to R. J. Nelson.

REFERENCES

1. **Ackerman KD, Madden KS, Livnat S, Felten SY, and Felten DL.** Neonatal sympathetic denervation alters the development of in vitro spleen cell proliferation and differentiation. *Brain Behav Immun* 5: 235–261, 1991.
2. **Bartness TJ, Demas GE, and Song CK.** Seasonal changes in adiposity: the roles of the photoperiod, melatonin and other hormones, and sympathetic nervous system. *Exp Biol Med* 227: 363–376, 2002.
3. **Bellinger DL, Lorton D, Felten SY, and Felten DL.** Innervation of lymphoid organs and implications in development, aging, and autoimmunity. *Int J Immunopharmacol* 14: 329–344, 1992.
4. **Bellinger DL, Lorton D, Lubahn C, and Felten DL.** Innervation of lymphoid organs—association of nerves with cells of the immune system and their implication in disease. In: *Psychoneuroimmunology* (3rd ed.), edited by Ader R, Felten DL, and Cohen N. New York: Academic, 2001, vol. 2.
5. **Benschop RJ, Rodriguez-Feuerhahn M, and Schedlowski M.** Catecholamine-induced leukocytosis: early observations, current research, and future directions. *Brain Behav Immun* 10: 77–91, 1996.
6. **Besedovsky HO, del Rey A, Sorkin E, DaPrada M, and Kelle HH.** Immunoregulation mediated by the sympathetic nervous system. *Cell Immunol* 48: 346–355, 1979.
7. **Brainard GC, Knobler RL, Podolin PL, Lavasa M, and Lubin FD.** Neuroimmunology: modulation of the hamster immune system by photoperiod. *Life Sci* 40: 1319–1326, 1987.
8. **Bronson FH and Heideman PD.** Seasonal regulation of reproduction in mammals. In: *The Physiology of Reproduction* (2nd ed.), edited by Knobil E and Neill JD. New York: Raven, 1994.
9. **Carlson SL, Beiting DJ, Kiani CA, Abell KM, and McGillis JP.** Catecholamines decrease lymphocyte adhesion to cytokine-activated endothelial cells. *Brain Behav Immun* 10:55–67, 1996.
10. **Champney TH and McMurray DN.** Spleen morphology and lymphoproliferative activity in short photoperiod exposed hamsters. In: *Role of Melatonin and Pineal Peptides in Neuroimmunomodulation,* edited by Franchini F and Reiter RJ. New York: Plenum, 1991.
11. **Cory AH, Owen TC, Barltrop JA, and Cory JG.** Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. *Can Commun Dis Rep* 3: 207–212, 1991.
12. **Demas GE and Bartness TJ.** Direct innervation of body fat and adrenal medullary catecholamines mediate photoperiodic changes in body fat. *Am J Physiol Regul Integr Comp Physiol* 281: R1499–R1505, 2001.
13. **Demas GE, Drazen DL, Jasnow AM, Bartness TJ, Nelson RJ.** Sympathoadrenal system differentially affects photoperiodic changes in humoral immunity of Siberian hamsters (*Phodopus sungorus*). *J Neuroendocrinol* 14: 29–35, 2002.
14. **Demas GE, Klein SL, and Nelson RJ.** Reproductive and immune responses to photoperiod and melatonin are linked in *Peromyscus* subspecies. *J Comp Physiol [A]* 179: 819–825, 1997.
15. **Demas GE and Nelson RJ.** Photoperiod and ambient temperature interact to affect immune parameters in male deer mice (*Peromyscus maniculatus*). *J Biol Rhythms* 11: 95–103, 1996.
16. **Dhabhar FS and McEwen BS.** Bidirectional effects of stress and glucocorticoid hormones on immune function: possible explanations for paradoxical observations. In: *Psychoneuroimmunology* (3rd ed.), edited by Ader R, Felten DL, and Cohen N. New York: Academic, 2001.
17. **Drazen DL, Bartness TJ, Nelson RJ, and Demas GE.** The sympathoadrenal system mediates photoperiodic changes in immune function in Siberian hamsters (*Phodopus sungorus*). *Soc Neurosci Abstr* 16: 2000.
18. **Drazen DL, Kriegsfeld LJ, Schneider JE, and Nelson RJ.** Leptin, but not immune function, is linked to reproductive responsiveness to photoperiod. *Am J Physiol Regul Integr Comp Physiol* 278: R1401–R1407, 2000.
19. **Elenkov IJ, Wilder RJ, Chrousos GP, and Vizi ES.** The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev* 52: 595–638, 2000.
20. **Friedman EM and Irwin MR.** Modulation of immune cell function by the autonomic nervous system. *Pharmacol Ther* 74: 27–38, 1997.
21. **Fuchs BA, Campell KS, and Munson AE.** Norepinephrine and serotonin content of the murine spleen: its relationship to lymphocyte β-adrenergic receptor density and the humoral immune response in vivo and in vitro. *Cell Immunol* 117: 339–351, 1988.
22. **Goldman BD.** Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. *J Biol Rhythms* 16: 283–301, 2001.
23. **Hadden JW, Hadden EM, and Middleton E.** Lymphocyte blast transformation. I. Demonstration of adrenergic receptors in human peripheral lymphocytes. *Cell Immunol* 1: 583–595, 1970.
24. **John JL.** The avian spleen: a neglected organ. *Q Rev Biol* 69: 327–351, 1994.
25. **Jones DD, Ramsay TG, Hausman GJ, and Martin RJ.** Norepinephrine inhibits rat pre-adipocyte proliferation. *Int J Obes Relat Metab Disord* 16: 349–354, 1992.
26. **Kouassi E, Li YS, Boukhris W, Millet I, and Revillard JP.** Opposite effects of the catecholamines dopamine and norepinephrine on murine polyclonal B-cell activation. *Immunopharmacology* 16: 125–137, 1988.
27. **Landmann R.** Beta-adrenergic receptors in human leukocyte subpopulations. *Eur J Clin Invest* 22, *Suppl* 1: 30–36, 1992.
28. **Livnat S, Felten SY, Carlso SL, Bellinger DL, and Felten DL.** Involvement of peripheral and central catecholamine systems in neural-immune interactions. *J Neuroimmunol* 10: 5–30, 1985.
29. **Lochmiller RL and Deerenberg C.** Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88: 87–98, 2000.
30. **Lochmiller RL, Vestey MR, and McMurray ST.** Temporal variation in humoral and cell-mediated immune responses in a *Sigmodon hispidus* population. *Ecology* 75236–75245, 1994.
31. **Lyte M, Ernst S, Driemeyer J, and Baissa B.** Strain-specific enhancement of splenic T cell mitogenesis and macrophage phagocytosis following peripheral axotomy. *J Neuroimmunol* 31: 1–8, 1991.
32. **Madden KS.** Catecholamines, sympathetic nerves, and immunity. In: *Psychoneuroimmunology* (3rd ed.), edited by Ader R, Felten DL, and Cohen N. New York: Academic, 2001, vol. 1.
33. **Madden KS, Sanders VM, and Felten DL.** Catecholamine influences and sympathetic neural modulation of immune responsiveness. *Annu Rev Pharmacol Toxicol* 35: 417–448, 1995.
34. **Malpaux B, Thiery JC, and Chemineau P.** Melatonin and the seasonal control of reproduction. *Reprod Nutr Dev* 39: 355–366, 1999.
35. **Mefford IN.** Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: measurement of catecholamines, serotonin and metabolites in rat brain. *J Neurosci Methods* 3: 207–224, 1981.
36. **Miles K, Quintans J, Chelmicka-Schorr E, and Arnason BG.** The sympathetic nervous system modulates antibody response to thymus-independent antigens. *J Neuroimmunol* 1: 101–105, 1981.
37. **Nelson RJ and Demas GE.** Seasonal changes in immune function. *Q Rev Biol* 71: 511–548, 1996.
38. **Nelson RJ, Demas GE, Klein SL, and Kriegsfeld LJ.** *Seasonal Patterns of Stress, Immune Function, and Disease.* Cambridge Univ. Press: New York, 2002.

39. **Nelson RJ, Fine JB, Demas GE, and Moffat CA.** Photoperiod and population density interact to affect reproductive and immune function in male prairie voles. *Am J Physiol Regul Integr Comp Physiol* 270: R571–R577, 1996.
40. **Rivkin I, Rosenblatt J, and Becker EL.** The role of cyclic AMP in the chemotactic responsiveness and spontaneous motility of rabbit perotoneal neutrophils. The inhibition of neutrophil movement and the elevation of cyclic AMP levels by catecholamines, prostaglandins, theophylline, and cholera toxin. *J Immunol* 115: 1126–1134, 1975.
41. **Rook GA.** Glucocorticoids and immune function. *Baillieres Best Pract Res Clin Endocrinol Metab* 13: 567–581, 1999.
42. **Sanders VM, Kasprowicz DJ, Kohm AP, and Swanson MA.** Neurotransmitter receptors on lymphocytes and other lymphoid cells. In: *Psychoneuroimmunology* (3rd ed.), edited by Ader R, Felten DL and Cohen N. New York: Academic, 2001, vol. 1.
43. **Sanders VM and Kohm AP.** Sympathetic nervous system interaction with the immune system. *Int Rev Neurobiol* 52: 17–41, 2002.
44. **Schedlowski M, Hosch W, Oberbeck R, Benschop RJ, Jacobs R, Raab HR, and Schmidt RE.** Catecholamines modulate human NK cell circulation and function via spleen-independent β-adrenergic mechanisms. *J Immunol* 156: 93–99, 1996.
45. **Stevens-Felten SY and Bellinger DL.** Noradrenergic and peptidergic innervation of lymphoid organs. *Chem Immunol* 69: 99–131, 1997.
46. **Viswanathan M, Hissa R, and George JC.** Suppression of sympathetic nervous system by short photoperiod and melatonin in the Syrian hamster. *Life Sci* 6: 73–79, 1986.
47. **Williams JM, Peterson RG, Shea PA, Schmedtje JF, Bauer DC, and Felten DL.** Sympathetic innervation of murine thymus and spleen: evidence for a functional link between the nervous and immune systems. *Brain Res Bull* 6: 83–94, 1981.
48. **Yellon SM, Fagoaga OR, and Nehlsen-Cannarella SL.** Influence of photoperiod on immune cell functions in the male Siberian hamster. *Am J Physiol Regul Integr Comp Physiol* 276: R97–R102, 1999.
49. **Youngstrom TG and Bartness TJ.** Catecholaminergic innervation of white adipose tissue in the Siberian hamster. *Am J Physiol Regul Integr Comp Physiol* 268: R744–R751, 1995.