Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, No. 4, pp. 821–825, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00040-2

Effects of Buspirone on the Immune Response to Stress in Mice[1]

MANUEL FREIRE-GARABAL,[2] MARÍA J. NÚÑEZ-IGLESIAS, JOSÉ L. BALBOA, JOSÉ C. FERNÁNDEZ-RIAL AND MANUEL REY-MÉNDEZ

NIMUS, Department of Pharmacology, School of Pharmacy, University of Santiago de Compostela. Campus Sur s/n. 15703-Santiago de Compostela, Spain

Received 15 June 1993

FREIRE-GARABAL, M., M. J. NÚÑEZ-IGLESIAS, J. L. BALBOA, J. C. FERNÁNDEZ-RIAL AND M. REY-MÉNDEZ. *Effects of buspirone on the immune response to stress in mice.* PHARMACOL BIOCHEM BEHAV **51**(4) 821–825, 1995.—Several experiments were conducted to evaluate the effects of buspirone, a selective 5-hydroxytryptamine-1A (5-HT_{1A}) anxiolytic, on the immune system of mice exposed to a chronic auditory stressor. Daily injection with 0.5 and 1 mg/kg (intraperitoneally) of buspirone resulted in a dose-dependent reduction in the stress-induced suppression of the natural killer (NK) cell activity and the in vitro and in vivo activity of phagocytosis. Higher doses of buspirone (2.0 mg/kg) showed less robust immunoenhancing effects in stressed mice, and caused a significant suppression of these immune parameters in unstressed mice.

Buspirone Stress Natural killer (NK) cell activity Phagocytosis

THE adverse effects of different schedules of stress on the number and functional capacities of T-cells in mice have been widely documented. Changes in the absolute number of lymphocytes, T-lymphocytes, and T-helper and T-suppressor cells of rodents have been reported (14,34). Stress also interferes with several immune responses such as murine splenic cytotoxic activity, both mediated by natural killer (NK) cells and cytotoxic T-lymphocytes (CTL) (3,19,23,24,28,31,34), the activity of phagocytosis (28), and the T-dependent antibody responses (20).

The effects of benzodiazepine agonist anxiolytics on the immune system have been widely documented. Benzodiazepines have been found partially to reverse the suppressive effects of different schedules of stress on the NK cell activity and in vivo and in vitro activity of phagocytosis (13,18). Furthermore, the protective effects of benzodiazepines against the adverse effects of stress on cancer (16) and infection (17) have been demonstrated.

Buspirone is an atypical anxiolytic drug that exerts its action at the receptor site other than GABA-benzodiazepine-chloride ionophore complex. Our preliminary data showed that buspirone partially reversed the suppressive effects of stress on T-cell populations and the blastogenic response of spleen cells to concanavalin A (Con A) (14). In this study we further investigated the effects of buspirone on the NK cell activity and in vitro and in vivo activity of phagocytosis in mice exposed to a chronic auditory stressor.

METHOD

Mice

Four-week-old inbred male BALB/c mice (Interfauna Ibérica S.A., Barcelona, Spain) were used. They were housed four per cage in well-ventilated rooms kept between 21 and 22°C and maintained on an alternating 12 L : 12 D cycle. Food (Panlab Diet A.03, Barcelona, Spain) and water were given ad lib.

Procedure

Mice were randomly dealed in four groups: group, unstimulated controls injected with placebo; group B, unstimulated mice injected with buspirone; group, stressed mice injected with placebo; and group D, stressed mice injected with buspirone. Six animals per group were sacrificed for each determina-

[1] These results were originally presented at the Second Annual International Behavioral Neuroscience Society Conference, held in Clearwater Beach, Florida, USA, from April 22–25, 1993.

[2] Reprint requests should be addressed to Dr. Manuel Freire-Garabal, C/ Dr. Teijeiro 30A-7oD, 15701 Santiago de Compostela, Spain.

tion after 1, 2, 4, 8, and 16 days of stress application. The time of decapitation and collection of samples was always < 5 min.

Induction of Stress

The mice were subjected to a broadband noise at about 100 dB daily for 5 s/min during 1- or 3-h periods around midnight, at the height of the diurnal activity cycle (23). Unstimulated controls were exposed only to the normal activity of the animal room.

NK Assay

Mice were sacrificed and their spleens were removed aseptically. Spleen cells were obtained by gentle teasing in RPMI-1640 medium (Gibco Laboratories, Grand Island, NY). The cell suspensions were filtered through a Nytex mesh (Flow Laboratories, Irvine, Ayrshire, UK) washed once with RPMI-1640. The pellets were then resuspended in culture medium (CM). The CM was RPMI-1640 supplemented with 10% fetal bovine serum (Gibco), 200 mM L-glutamine (Gibco), 26 mM Hepes (Gibco), and 50 μg/ml gentamycin (Sigma Chemical Co., St Louis, MO.

YAC-1 cells (ECACC, Salisbury, UK) were used as the target in the chromium-51 release assay. A suspension of 5 × 106 YAC-1 cells in 0.9 ml CM was labeled with 100 μCi of sodium chromate-51 (Incstar Co., Madrid, Spain) for 60 min at 37°C in a 5% CO_2 incubator. Cells were then washed three times in RPMI-1640 and resuspended in the complete CM at the concentration of 1 × 105 cells/ml.

The NK cell activity of murine effector cells was individually estimated by 4-h chromium-51 release assay according to the procedures of Favalli et al. (14). Effector cells were adjusted to varying concentrations and added to 1 × 104 chromium-51-labeled YAC-1 cells in U-shaped 96-well microtiter plates (Flow laboratories) in a total volume of 0.2 ml After incubation for 4 h at 37°C in a 5% incubator, the plates were centrifuged at 350 × *g* for 10 min; 0.1-ml samples of supernatant were collected and their radioactivity was measured (LKB Wallac 1275 γ-counter, Barcelona, Spain). Assays were done in quadruplicate and the baseline cromium-51 release never exceeded 105 of the total counts incorporated by target cells. Results are expressed as cytotoxicity obtained at three E/T ratios. One LU corresponded to the number of effector cells required to produce 20% specific lysis. Specific cytotoxicity was calculated as follows (12): SC = Test counts − (Baseline counts/min)/(Total counts/min incorporated) − (Baseline counts/min).

In Vitro Phagocytosis of Macrophages

Macrophages were collected by washing the peritoneal cavity with ice-cold PBS (0.1 M, pH 7.3). The peritoneal cells were washed twice with RPMI culture medium (Gibco) by centrifugation (1000 rpm, 5 min, 0°C) and finally resuspended in an adequate volume of RPMI medium supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco) to give a concentration of 1 × 106 nucleated cells/ml. Aliquots (1 ml) of this suspension were seeded in 35-mm Petri dishes and incubated at 37°C in a humidified 5% CO_2 and 95% air incubator. Three hours later, nonadherent cells were washed off and 1 ml fresh medium containing 10% FCS was added. Zymosan was added to give 5 × 106 particles/ml, and incubation was continued. Thirty minutes later, particle uptake was measured by light microscopy. Cells containing three or more zymosan particles were counted as phagocytic (28).

In Vivo Phagocytosis of Macrophages

Phagocytic activity was estimated by using the carbon clearance test (28). Carbon suspension (Pelikan c11/1432a, Madrid, Spain) was centrifuged at 5000 rpm for 15 min, and the supernatant was diluted threefold with sterile 1.5% gelatin saline to bring the carbon concentration to about 30 mg/ml. Diluted carbon suspension was injected at 0.1 ml/10 g body wt. into the tail vein of mice. After 0.5 and 10 min of injection, a blood sample (0.05 ml) was collected by puncturing the retro-orbital venous plexus. Blood was hemolyzed by the addition of 1 ml 0.1% Na_2CO_3 solution, followed by measurement of its optical density at 600 nm. The phagocytic index, K, was derived from the following equation (28): $K = 1/(t_{10} - t_{0.5}) \log C_{0.5}/C_{10}$, where $C_{0.5}$ and C_{10} express the carbon concentration at times $t_{0.5}$ and t_{10}, respectively.

Drug Treatments

Buspirone was injected IP at different dose ratios (0.25, 0.5, 1.0, and 2 mg/kg) in a volume of 1 ml/kg of vehicle. Control-unstressed and control-stressed mice were injected with 1 ml/kg of vehicle as placebo. Drugs were administered daily at 0930 h 15 days before, because of the delayed appearance of the drug's effects (14), and during the whole period of stress application.

Statistical Analysis

Statistical analysis was performed using the Student's *t*-test, χ^2 analysis, or one-way analysis of variance with grouping of means by Fisher's least-significant difference method.

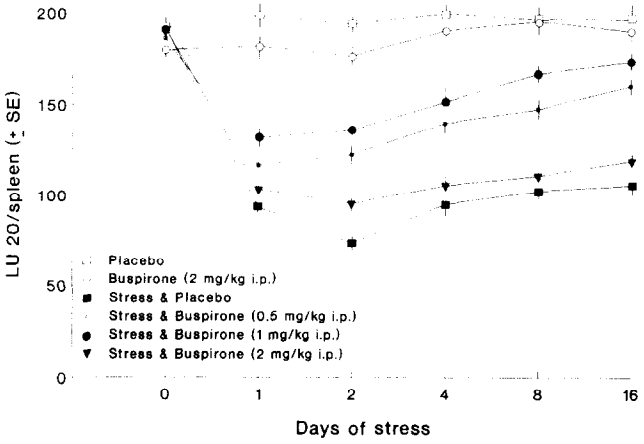

FIG. 1. NK-cell activity of spleen cells collected from stressed mice injected with placebo or buspirone. Lytic units (LU) per spleen were calculated by linear regression analysis. One LU corresponded to the number of effector cells required to produce 20% specific lysis. Values represent the mean values ± SE of three assays per spleen in six mice. LU_{20}/spleen values for unstimulated controls were 215.7 ± 17.2. Differences between stressed mice injected with placebo and stressed mice injected with 0.5 and 1 mg/kg buspirone were significant. Differences between stressed mice injected with placebo and stressed mice injected with 2.0 mg/kg buspirone were not significant except on the 2nd day after stress application. Unstressed mice injected with 2 mg/kg buspirone showed a significant decrease in the NK-cell activity until the 2nd day of assays. Significance was achieved at $p < 0.05$.

RESULTS

Natural killer cytotoxicity, expressed as LU_{20}/spleen ± SE, was reduced in stressed mice injected with placebo in comparison with unstressed controls (Fig. 1). Treatment with 0.5 and 1 mg/kg buspirone resulted in a dose-dependent reduction of the stress-induced suppression of the NK cell activity. By contrast, higher doses of buspirone (2 mg/kg) did not significantly affect the NK cell activity of stressed mice, unless on the 2nd day after stress application. Unstressed controls injected with 2 mg/kg buspirone only showed a significant decrease in the NK cell activity until the 2nd day of assays.

Figure 2 shows the results of the in vitro phagocytic activity of peritoneal macrophages. Although the same number of macrophages were cultured in all experimental groups, the number of macrophages containing zymosan particles were significantly reduced in stressed mice injected with placebo. Treatment with 0.5 and 1 mg/kg buspirone also reduced the suppressive effects of stress in a dose-dependent manner; meanwhile, higher doses of this compound (2 mg/kg) only reduced the suppressive effects of stress after 2 and 4 days of stress application. Treatment with 2 mg/kg buspirone reduced the percentage of particle uptake of macrophages in unstressed mice.

In vivo studies also showed a decrease of the carbon clearance in stressed mice (Fig. 3). The PI was also promoted in a dose-dependent manner by the administration of 0.5 and 1 mg/kg buspirone. By contrast, lower protective effects were observed after the administration of 2 mg/kg of this compound, unless on the 1st day after stress application, when no differences were appreciated between placebo and buspirone. Suppressive effects of 2 mg/kg buspirone in unstressed mice were also observed.

DISCUSSION

We observed that stress powerfully inhibited natural and specific cellular immune responses. A significant reduction of NK cell activity was observed during all the period of stress application in comparison with unstressed controls. The in vitro and in vivo activity of phagocytosis was also affected. Our results are in good agreement with those previously reported by other authors (3,19,23,24,28,31,34).

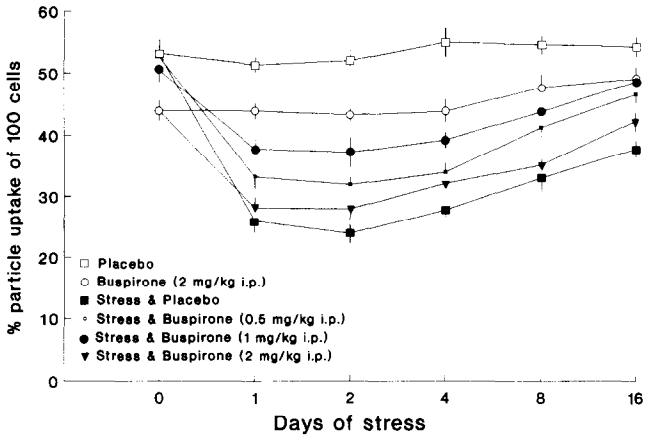

FIG. 2. In vitro activity of phagocytosis. The results represent the mean ± SD of six animals. Differences between groups were significant at $p < 0.01$. Treatment with 0.5 and 1 mg/kg buspirone was also found to reduce the suppressive effects of stress in a dose-dependent manner; higher doses of this compound (2 mg/kg) only reduced the suppressive effects of stress on the 2nd day of stress application. Treatment with 2 mg/kg buspirone reduced the percentage of particle uptake of macrophages in unstressed mice. Differences were considered significant at $p < 0.05$.

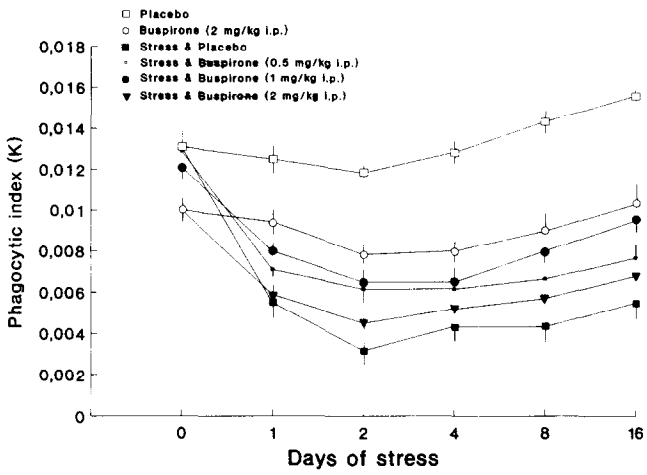

FIG. 3. Effect of buspirone on carbon clearance activity in stressed mice. The results represent the mean ± SD of six animals. Treatment with 0.5 and 1 mg/kg buspirone resulted in a dose-dependent reduction of stress-induced suppression of NK-cell activity. The 2-mg/kg dose of buspirone did not significantly affect the PI except on the 2nd day of stress application. Suppressive effects of 2 mg/kg buspirone in unstressed mice were observed. Significance was achieved at $p < 0.05$.

The mechanism by which stress inhibits the cellular immune response has been widely studied. A molecular basis for bidirectional communication between the immune and neuroendocrine systems has been described (7,8). Cell-to-cell communications between the immune and the neuroendocrine systems are primarily mediated by hormones and neuropeptides that reach lymphoid organs and cells through the vascular system or directly through the autonomic connections between nerve endings and lymphoid organs (1,11). Receptor sites are present in lymphoid cells for many hormones and neurotransmitters (9,38). In this respect, a number of molecules produced by cells of the nervous system such as ACTH, PRL, opioid peptides, GH, TSH, AVP, CRH, dynorphin, dopamine, and others have been found to modulate immune functions.

On the other hand, humoral factors generated by the immune system such as thymic peptides and lymphokines modulate neuroendocrine functions. In addition, in the course of lymphocyte activation, lymphoid cells may produce hormonal substances very much like those produced by the hypophysis, such as ACTH, TSH, GH, PRL, gonadotrophin, and β-endorphin (6,9,38,39).

Treatment with buspirone affected the immune response to stress. Dose-related protective effects of this compound on the NK cell and the phagocytic activities of stressed mice were found when administered at 0.5–1 mg/kg, whereas if administered at higher doses (2.0 mg/kg), this effect was significantly smaller. In some cases, there were no significant differences in comparison with stressed mice injected with placebo. Furthermore, suppressive effects of buspirone on the NK cell activity and on the in vitro and in vivo activity of phagocytosis were found when injected at 2.0 mg/kg in unstressed mice.

In comparison with benzodiazepines, the effects of buspir-

one on the immune system are lower in intensity and delayed in time. As shown in a previous experiment (14), it is necessary to initiate drug administration 15 days before the exposure to the stressor to observe differential effects on immune parameters.

The mechanism of action of buspirone on the immune system may be either direct or indirect. Buspirone is a 5-HT_{1A} agonist anxiolytic with moderate affinity for D_2 and σ-receptors (22). It is also important to note that 1-(2-pyrimidinyl)-piperazine (1-PP), the metabolite of buspirone, has potent α_2-antagonist activity (5).

Although direct effects of buspirone (at target cell) should not be excluded, one can hypothesize that the regulation of the immune response may result from a mediator involved in expressing the drug's effect. The role of 5-HT_{1A} receptors and the potential agonists in the regulation of hormone secretion is beginning to be determined. Buspirone has been found to increase plasma ACTH and corticosterone-cortisol concentrations in rodents and humans (22). In addition, 1-PP increases corticosterone secretion. Buspirone also decreases GH (33) and TSH secretion (10). These data are in accordance with our present results on the suppressive effects of the 2.0-mg/kg dose of buspirone on the immune system of unstressed mice.

By contrast, the ability of buspirone to reduce the stress elevation of hormone secretion has not been clearly established (22). In a previous report, we observed that the protective effects of 1 mg/kg buspirone on T-cell immune responses were accompanied by a proportional reduction in ACTH levels of stressed mice (14). Because a rise in plasma corticosterone concentrations, via ACTH secretion, has an easily demonstrable destructive effect on specific cells and tissues that are required for optimum immunologic defense (2,21,25,29, 30,32,35), the buspirone-induced decrease in ACTH levels should partially help to explain the immunoprotective effects of this drug. Interestingly, other authors (22) found that the stress-induced increase in corticosterone secretion was inhibited by the 0.5-mg/kg (IP) dose but not by the 2.0 mg/kg (IP) dose of buspirone, which increased corticosterone secretion in stressed rats.

Other neuroendocrinologic effects of buspirone in stressed mice should affect the immune system. For example, buspirone has been found to enhance PRL secretion in unstressed rodents (27) but produces a dose-dependent attenuation of the stress-induced increase in PRL secretion (5,22,36). Prolactin has well-known immunoenhancing effects. Chemical blockade of PRL release has been found to abrogate T-lymphocyte-dependent activation of macrophages as well as the production of lymphocyte interferon following inoculation with listeria or mycobacteria (4), and suppresses T-lymphocyte proliferation, delayed cutaneous hypersensitivity, and the in vivo tumoricidal activation of peritoneal macrophages (26). Therefore, many questions still need to be addressed to fully understand the pathways by which buspirone may regulate the immune response to stress.

In recent years, there has been a growing concern regarding the effects of stress on cancer. Because it has been pointed out that reduced cell-mediated immunity may adversely affect cancer (28), our present data are consistent with the hypothesis of the beneficial effects of buspirone on the host surveillance to destroy the transformed malignant cells during their immunologically vulnerable stage. In this regard, our previous studies showed beneficial effects of buspirone against the facilitatory response of MTV-induced tumors to stress (15). Nevertheless, biologic significance and health relatedness should be assessed.

ACKNOWLEDGEMENTS

The authors express their gratitude to J. A. Veira and J. M. Rubinos for their technical support.

REFERENCES

1. Ader, R.; Felten, D.; Cohen, N. Interactions between the brain and the immune system. Annu. Rev. Pharmacol. Toxicol. 30: 561-602; 1990.
2. Bassett, J. R.; Cairncross, K. D. Effects of psychoactive drugs on responses of the rat to adversive stimulation. Archs. Int. Pharmacodyn. Ther. 212:221-229; 1974.
3. Ben-Eliyahu, S.; Yirmiya, R.; Shavit, Y.; Liebeskind, J. C. Stress-induced suppression of natural killer cell cytotoxicity in the rat: A naltrexone-insensitive paradigm. Behav. Neurosci. 104: 235-238; 1990.
4. Bernton, E. W.; Bryant, H. U.; Holaday, J. W. Prolactin and immune function. In: Ader R.; Felten D. L.; Cohen N., eds. Psychoneuroimmunology. San Diego, California: Academic Press; 1991:403-428.
5. Bianchi, G.; Caccia, S.; Della Vedova, F.; Garattini, S. The alpha 2-adrenoceptor antagonist activity of ipsapirone and gepirone is mediated by their common metabolite 1-(2-pyrimidinyl)-piperazine (PmP). Eur. J. Pharmacol. 151:365-371; 1988.
6. Blalock, J. E. Proopiomelanocortin-derived peptides in the immune system. Clin. Endocrinol. 22:823-827; 1985.
7. Blalock, J. E. A molecular basis for bidirectional communication between the immune and neuroendocrine systems. Physiol. Rev. 69:1-32; 1989.
8. Blalock, J. E. Molecular mechanisms of bidirectional communication between the immune and neuroendocrine systems. Int. J. Neurosci. 51:363-364; 1990.
9. Blalock, J. E.; Bost, K. L.; Smith, E. M. Neuroendocrine peptide hormones and their receptors in the immune system. Production, processing and action. J. Neuroimmunol. 10:31-40; 1985.
10. Broqua, P.; Baudrie, V.; Bluet-Pajot, M. T.; Chaouloff, F. Buspirone, ipsapirone and 1-(2-pyrimidinyl)-piperazine decrease cold-induced thyrotropin secretion in rats. Eur. J. Pharmacol. 204:141-147; 1991.
11. Bullock, K. The innervation of immune system tissues and organs. In: Cotman C. W.; Brinton R. E.; Galaburda A.; McEwen B., eds. The neuro-immune-endocrine connection. New York: Raven Press; 1987:33-47.
12. Di Francesco, P.; Pica, F.; Croce, C.; Favalli, C.; Tubaro, E; Garaci, E. Effect of acute or daily cocaine administration on cellular immune response and virus infection in mice. Nat. Immun. Cell Growth Regul. 9:397-405; 1990.
13. Freire-Garabal, M.; Belmonte, A.; Balboa, J. L.; Núñez, M. J. Effects of midazolam on T-cell immunosupressive response to surgical stress in mice. Pharmacol. Biochem. Behav. 43:85-89; 1992.
14. Freire-Garabal, M.; Belmonte, A.; Suárez-Quintanilla, J. A. Effects of buspirone on T-cell immunosuppressive response to stress in mice. Arch. Int. Pharmacodyn. Ther. 314:160-168; 1991.
15. Freire-Garabal, M.; Núñez, M. J.; Balboa, J. L.; Belmonte-Otero, A.; Belmonte, A. Effects of buspirone on the development of MTV-induced mammary tumors in female mice under stress. Res. Commun. Psych. Psy. 16:107-114; 1991.
16. Freire-Garabal, M.; Núñez, M. J.; Balboa, J. L.; Suárez, J. A.; Belmonte, A. Effects of alprazolam on the development of MTV-induced mammary tumors in female mice under stress. Cancer Lett. 62:185-189; 1991.
17. Freire-Garabal, M.; Núñez, M. J.; Fernández-Rial, J. C.; Balboa, J. L.; Couceiro, J.; Belmonte, A. Effects of alprazolam on

influenza virus infection in stressed mice. Pharmacol. Biochem. Behav.; in press.
18. Fride, E.; Skolnick, P.; Arora, P. K. Immunoenhancing effects of alprazolam in mice. Life Sci. 47:2409–2420; 1990.
19. Irvin, J.; Livnat, S. Behavioral influences on the immune system: Stress and conditioning. Prog. Neuropsychopharmacol. Biol. Psychiatry 11:137–143; 1987.
20. Keller, S. E.; Schleifer, S. J.; Liotta, A. S.; Bond, R. N.; Farhoody, N.; Stein, M. Stress-induced alterations of immunity in hypophysectomized rats. Proc. Natl. Acad. Sci. 85:9297–9301; 1988.
21. Le Fur, G.; Guilloux, F.; Mitrani, N.; Mizoule, J.; Uzan, A. Relationships between plasma corticosteroids and benzodiazepines in stress. J. Pharmacol. Exp. Ther. 211:305–308; 1979.
22. Levy, A. D.; Van de Kar, D. Endocrine and receptor pharmacology of serotoninergic anxiolytics, antipsychotics and antidepressants. Life Sci. 51:83–94; 1992.
23. Monjan, A. A.; Collector, M. I. Stress-induced modulation of the immune response. Science. 15:307–308; 1977.
24. Morley, J. E.; Kay, N. E.; Solomon, G. F.; Plotnikoff, N. P. Neuropeptides: Conductors of the immune orchestra. Life Sci. 41:527–544; 1987.
25. Munck, A.; Guyre, P. M. Glucocorticoid physiology, pharmacology and stress. Adv. Exp. Med. Biol. 196:81–96; 1986.
26. Nagy, E.; Berezi, J.; Wren, G.; Asa, S.; Kovacs, K. Immunomodulation by bromocriptine. Immunopharmacology 6:231–243; 1983.
27. Nash, J. F.; Meltzer, H. Y. Effect of gepirone and ipsapirone on the stimulated and unstimulated secretion of prolactin in the rat. J. Pharmacol. Exp. Ther. 249:236–241; 1989.
28. 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.
29. Riley, V. Psychoneuroendocrine influences on immunocompetence and neoplasia. Science 212:1100–1109; 1981.
30. Schultz, R. M.; Chirigos, M. A.; Stoychkov, J. N.; Pavlidis, N. A. Factors affecting macrophage cytotoxic activity with particular emphasis on corticosteroids and acute stress. J. Reticuloendoth. Soc. 26:83–92; 1979.
31. Shavit, Y.; Terman, G. W.; Martin, F. C.; Lewis, J. W.; Liebeskind, J. C.; Gale, R. P. Stress, opioid peptides, the immune system and cancer. J. Immunol. 135(Suppl. 2):834–837; 1985.
32. Solomon, G. F.; Merigan, T. C.; Levine S. Variation in adrenal cortical hormones within physiological ranges, stress and interferon production in mice. Proc. Soc. Exp. Biol. Med. 126:74–79; 1967.
33. Spencer, G. S.; Dobbie, P.; Bass, J. J. Neuroendocrine regulation of growth hormone secretion in sheep. III. Serotoninergic systems. Domest. Anim. Endocrinol. 8:383–91; 1991.
34. Steplewski, Z.; Goldman, P. R.; Ehya, H.; Poropatich, C.; Smith, J. M. Effects of restraint stress on inoculated tumor growth and immune response in rats. Cancer Res. 45:5128; 1985.
35. Torres-Aleman, I.; Barasoain, I.; Borrell, J.; Guaza, C. Immune activation and psychoneurogenic stress modulate corticosterone-releasing effects of lymphokines and ACTH. Am. J. Physiol. 255: 839–845; 1988.
36. Urban, J. H.; Van de Kar, L. D.; Lorens, S. A.; Bethea, C. L. Effect of the anxiolytic drug buspirone on prolactin and corticosterone secretion in stressed and unstressed rats. Pharmacol. Biochem. Behav. 25:457–462; 1986.
37. Vogel, W. H.; Bower, D. B. Stress, immunity and cancer. In: Plotnikoff, N.; Murgo, A.; Faith, R.; Wybran, J., eds. Stress and immunity. Boca Raton, FL: CRC Press; 1991:493–507.
38. Weigent, D. A.; Blalock, J. E. Immunoreactive growth hormone releasing hormone production by rat leukocytes. J. Neuroimmunol. 29:1–13; 1990.
39. Weigent, D. A.; Carr, D. J.; Blalock, J. E. Bidirectional communication between the neuroendocrine and the immune systems. Common hormones and hormone receptors. Ann. NY Acad. Sci. 579:17–27; 1990.