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Stressor-Induced Alterations of Natural Killer Cell Activity and Central Catecholamines in Mice

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ZALCMAN, S., J. IRWIN AND H. ANISMAN. *Stressor-induced alterations of natural killer cell activity and central catecholamines in mice.* PHARMACOL BIOCHEM BEHAV **39**(2) 361–366, 1991.—Natural killer (NK) cell cytotoxicity was determined at various intervals (0.5, 24 or 48 h) following exposure to uncontrollable footshock in 3 strains of mice. Stressor application provoked reductions of NK activity, but the time course of the NK changes varied across strains. Whereas NK cytotoxicity was markedly reduced in C57BL/6J mice 0.5–48 h following stressor exposure, this effect was delayed in C3H/HeJ mice, being evident 24–48 h following stressor application. In BALB/cByJ mice, NK activity was significantly reduced 24 h after footshock, but in contrast to the other strains returned to control levels within 48 h of stressor exposure. Central NE and DA concentrations and activity were influenced by the stressor treatment in a strain-dependent fashion. However, the relationship between the central amine variations and the alterations of NK cytotoxicity associated with the stressor was limited.

Stress Natural killer cell cytotoxicity Norepinephrine Dopamine

IT has been demonstrated that environmental or psychological stressors may compromise immune functioning (7). Among other things, stressors, such as footshock and restraint, may reduce the plaque-forming cell (PFC) response and antibody titers to sheep red blood cells (SRBC) (8, 19, 33, 34), diminish lymphocyte proliferation in response to T- and B-cell mitogens (15, 18), and alter splenic natural killer cell (NK) cytotoxicity (13, 28, 31), as well as increase vulnerability to pathogens (7). Likewise, it has been reported that stressors provoke alterations in the turnover or levels of central neurotransmitters, including norepinephrine (NE), dopamine (DA), serotonin (5-HT) and endorphins (1, 22, 32).

While stressors reliably alter central amine activity and disrupt performance in several behavioral paradigms, it seems that appreciable interindividual and interstrain differences exist in this respect (3, 25, 26). Likewise, the impact of stressors on vulnerability to immunologically related illnesses may vary across strains of animals. For instance, strain differences were reported with respect to the number of cells producing antibodies to SRBC, as well as lymphocyte proliferation in response to concanavalin A. Moreover, it appeared that the effects of individual vs. group housing varied with the strain of mouse examined (21). It has similarly been reported that housing condition influenced vulnerability to encephalomyocarditis virus, and this effect varied across strains of mice (10). Finally, it has been reported that, soon after stressor exposure, splenic NK cytotoxicity varied in two strains of mice (17). It was thus of interest to further evaluate NK cytotoxicity in response to a stressor in different strains of mice. Moreover, since the potential impact of a stressor may vary over time, NK cell activity in independent groups was evaluated 0.5, 24 and 48 h after stressor application.

It has been demonstrated that central catecholamine activity and NK cytotoxicity may be affected by similar manipulations, such as stressor controllability and chronicity (1). Moreover, it appears that hypothalamic neurotransmitter functioning may influence splenic immune activity (5, 9, 16), and conversely, immunological manipulations may affect hypothalamic NE activity (2, 4). While central CRF and interleukin-1 as well as opioids have been posited to contribute to stressor-induced alterations of splenic NK cytotoxicity (6, 13, 29, 31), it is premature to dismiss a role for central catecholamines in this respect. Accordingly, it was of interest to determine whether a relationship existed between stressor-induced central catecholamine alterations and variations in splenic NK activity. Given the strain-specific effects of stressors on central neurotransmitter activity (3,26), in the present investigation we determined whether stressor-provoked variations of central amines were related to strain-dependent alterations of NK cytotoxicity induced by a stressor. To this end, concentrations of NE and DA, and their metabolites, 3-methoxy, 4-hydroxy-phenylethylene glycol (MHPG) and dihydroxyphenylacetic acid (DOPAC), respectively, were assessed in various brain regions 0.5 h after stressor administration and were related to the variations of NK cell cytotoxicity. Earlier studies had revealed that the impact of stressors of moderate severity persisted for only a matter of a few hours, and thus catecholamine activity was only assessed at this one time frame (1,32).

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METHOD

Subjects

A total of 104 experimentally naive, male mice of 3 inbred strains (BALB/cByJ, C3H/HeJ, C57BL/6J) were obtained from the Jackson Laboratories, Bar Harbor, ME, at 6 weeks of age. Mice were allowed a 7–14-day acclimatization period prior to being used as experimental subjects. Mice were housed in groups of 5 in standard polypropylene cages, maintained on a 12-hour light/dark cycle, and permitted ad lib access to food and water.

Apparatus

Four identical black Plexiglas chambers, measuring $30 \times 14 \times 15$ cm, were employed to deliver inescapable footshock. The chamber floors consisted of 0.32 cm stainless steel rods spaced 1.0 cm apart (center to center) and were connected in series by neon bulbs. In addition, the end walls of the chambers were lined with stainless steel plates which were connected in series with the grid floor. The lid of each box consisted of red translucent Plexiglas providing limited illumination. Footshock (150 μA, 60 Hz, AC) was delivered through a 3000-V source, thereby providing a relatively constant current.

Procedure

Mice were individually placed in the shock boxes and exposed to 360 shocks of 2-s duration (150 μA; 9-s intershock interval) or were not shocked. Shocked animals were returned to their home cages and placed in a holding room for either 0.5, 24 or 48 h. Immediately following these intervals, animals were sacrificed, and spleens were removed for subsequent determination of NK cell cytotoxicity. Spleens were placed in 15 ml conical tubes filled with Hanks Balanced Salt Solution (HBSS) and kept on ice for up to 4 h before the NK assay was initiated. Among mice decapitated 0.5 h after shock and among nonshocked animals, the brains were removed and dissected on slides maintained on dry ice. A micropunch technique was used to remove the nucleus accumbens, prefrontal cortex, locus coeruleus and dorsal hippocampus. The hypothalamus was excised in full. Tissue was stored at −60°C for subsequent HPLC determinations with coulometric detection of NE, DA and their metabolites, MHPG and DOPAC, using a slight modification of the procedure of Seegal, Brosch and Bush (24). It is well established that stressors provoke the most pronounced NE changes in the hypothalamus and in the dorsal bundle (locus coeruleus, as well as the hippocampus and frontal cortex), while DA alterations are most pronounced in the mesocortex and nucleus accumbens (1). Accordingly, only these regions were included in the NE and DA determinations.

NK cytotoxicity was assessed using a 6-h ^{51}Cr release assay. Spleen cell suspensions were prepared and incubated for 5 min in a buffer containing 0.155 M NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM EDTA in order to lyse red blood cells. The spleen cells were then washed three times in Hank's Balanced Salt Solution (Gibco). Cells were resuspended in RPMI-1640 culture medium (Gibco), at a concentration of 16×10^6 cells/ml from which serial dilutions were subsequently prepared. YAC-1 lymphoma cells, maintained in culture, were used as the target cells. YAC-1 cells were labelled by first washing them in RPMI-1640 and then incubating with 200 μCi $NaCrO_4$ (Amersham) at 37°C in a water bath for 1.5 h. Target cells were then washed 3 times in RPMI-1640 (10% FCS) and were resuspended at a final concentration of 10^5 cells/ml. Triplicate samples (0.1 ml) of individual spleen cell suspensions were placed together with target cells (10^4 cells in 0.1 ml) in 96-well round-bottomed microtiter plates (Flow Laboratories) at 4 effector:target ratios (160:1, 80:1, 40:1, 20:1). Additional wells containing targets and either RPMI-1640 (spontaneous release) or 0.1% nonidet detergent (maximum release) served as controls. The plates were centrifuged ($500 \times g$) for 5 min and then incubated for 6 h at 37°C in a humidified CO_2 chamber. Plates were then centrifuged for 5 min at 4°C, and supernatants (0.1 ml) were harvested into 6×50-mm tubes (Luckham) for counting in a gamma spectrometer. Percent cytotoxicity was calculated at each effector:target ratio, and these values were converted to lytic units at 30% lysis (LU_{30}) according to the method of Pross et al. (20).

RESULTS

The activity of NK cells was calculated as a percentage of the lytic units (LU_{30}) for each subject relative to the nonshocked animals of the same strain (Fig. 1). The $LU_{30}/10^7$ cells were 6.67 ± 0.95, 4.91 ± 0.24 and 8.11 ± 1.26 of nonstressed C57BL/6J, C3H/HeJ and BALB/cByJ mice, respectively. Since NK cytotoxicity was assayed independently for each of the strains, separate analyses of variance were conducted to determine the effects of the stressor on NK activity in these strains. It should be underscored that, since NK activity was assayed separately in each of the strains, comparisons were limited to the time-dependent effects of the stressor on NK activity within each strain. Analysis of variance of the NK activity in C57BL/6J mice yielded a significant effect of the shock treatment, $F(3,41) = 4.21$, $p<0.05$. Newman-Keuls multiple comparisons ($\alpha = 0.05$) revealed that the stressor provoked a marked reduction of NK activity at each of the intervals. As seen in Fig. 1, there was no indication of NK recovery occurring over the 48-h period. In C3H/HeJ mice, performance was again found to vary with the stressor treatment, $F(3,27) = 5.13$, $p<0.05$. The multiple comparisons indicated that NK activity was reduced at the 24- and 48-h intervals, but, at the 30-min interval, NK activity did not differ from that of nonshocked mice. Finally, in BALB/cByJ mice, the variations of NK activity induced by shock approached but did not reach statistical significance, $F(3,24) = 2.74$, $p = 0.066$. A posteriori comparisons which were nonetheless conducted revealed that, at 0.5 h following stressor exposure, the reduction of NK cytotoxicity was not significant. At the 24-h poststressor period, NK cytotoxicity was reduced relative to nonstressed animals but returned to control values at the 48-h interval. Thus, in BALB/cByJ mice, the reduction of NK activity was somewhat slower to develop and persisted for a shorter period of time.

Concentrations of NE, DA, MHPG and DOPAC as functions of the strain and shock treatments are shown in Table 1. Since HPLC determinations were conducted over several replications with tissue from all strains equally represented within each replication, data were converted to the percent of control values for each replication. Several tissue samples were lost during the course of the HPLC analyses, and, consequently, the degrees of freedom varied across brain regions. Analysis of variance of hypothalamic MHPG accumulation revealed that the shock treatment significantly increased concentrations of the metabolite, $F(1,39) = 15.88$, $p<0.01$. Analysis of the hypothalamic NE concentrations revealed a significant strain × shock treatment interaction, $F(2,41) = 4.84$, $p<0.05$. Newman-Keuls multiple comparisons ($\alpha = 0.05$) confirmed that the shock treatment reduced NE levels in C57BL/6J mice, produced a modest, nonsignificant decline in BALB/cByJ mice, and was without effect in C3H/HeJ mice. As in the present report, it was observed previously that the NE alterations were greater in C57BL/6J than in

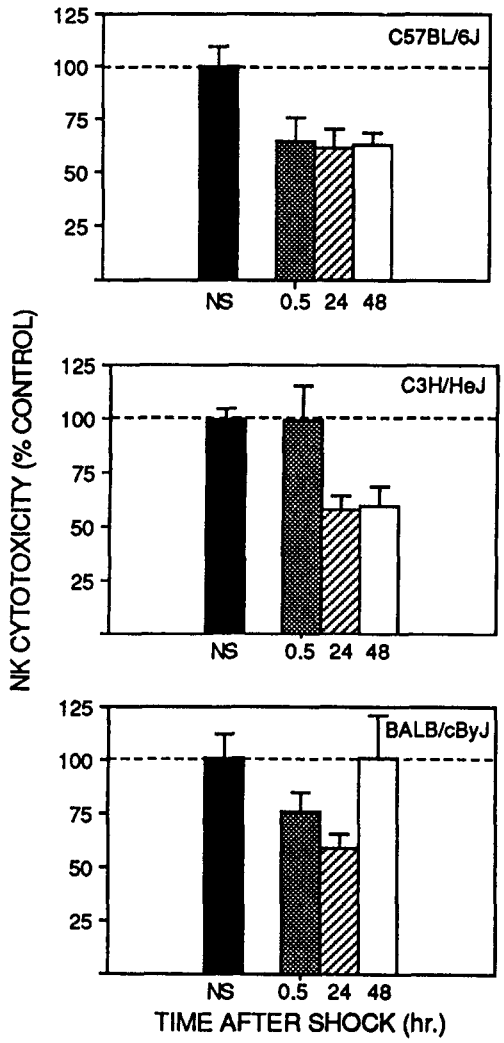

FIG. 1. Mean (+S.E.M.) NK cell cytotoxicity as a percent of control values in three strains of mice that were either not exposed to footshock (NS) or at various intervals after footshock (0.5, 24 or 48 h).

C3H/HeJ mice (26). However, in the earlier study, the hypothalamic NE reduction in BALB/cByJ mice was more pronounced than that in the present investigation. This between-experiment variability might conceivably be related to differences in the stressor severity employed in the two studies, or a result of tissue being taken 30 min after stressor termination in the present study instead of immediately following footshock, as in the previous report.

The accumulation of MHPG in the locus coeruleus was increased by the shock treatment, $F(1,39)=13.30$, $p<0.01$, whereas concentrations of NE were reduced, $F(1,40)=8.49$, $p<0.01$. While it appeared that the increase of MHPG was somewhat different across the strains, the MHPG values were relatively variable. As in the other brain regions, hippocampal MHPG accumulation was increased by the shock treatment, $F(1,43)=2.84$, $p<0.05$. While the strain × shock treatment interaction did not reach statistical significance, it is clear from Table 1 and confirmed by Newman-Keuls multiple comparisons ($\alpha=0.05$) that the shock treatment, in fact, did not increase hippocampal MHPG in C3H/HeJ mice. This finding is consistent with our earlier report showing a relatively reduced sensitivity of NE utilization in the hippocampus of this strain (26). Analysis of NE concentrations revealed that the shock treatment reduced concentrations of the amine, $F(1,43)=4.30$, $p<0.05$. The shock treatment × strain interaction approached but did not reach statistical significance, $F(2,43)=2.92$, $p=0.06$. Newman Keuls multiple comparisons of the means comprising this interaction ($\alpha=0.05$) nevertheless indicated that, in C57BL/6J mice, NE levels were significantly reduced by the stressor, while, in BALB/cByJ mice, the decline did not reach statistical significance. There was no evidence of an NE reduction in shocked C3H/HeJ mice. Finally, mesocortical MHPG accumulation was markedly increased by the stressor, $F(1,37)=8.30$, $p<0.01$. The extent of the increase did not vary as a function of the strain, although once again the effect appeared to be less pronounced, although not significantly so, in the C3H/HeJ mice than in the remaining strains. Mesocortical NE concentrations were not significantly affected by the stressor, although it was observed that, in the BALB/cByJ and C57BL/6J strains, NE was reduced by more than 20% by the shock treatment.

The variations of DA and DOPAC associated with the stressor in each of the strains is shown in Table 1. Analysis of variance of DOPAC accumulation in the nucleus accumbens revealed that the shock treatment increased the metabolite accumulation, $F(1,34)=4.03$, $p=0.05$. The shock treatment × strain interaction approached but did not reach statistical significance, $F(2,34)=2.93$, $p=0.07$. Nevertheless, Newman-Keuls multiple comparisons were conducted on the basis of a priori predictions that had been made concerning this interaction. These comparisons revealed that, in BALB/cByJ mice, DOPAC was markedly increased by shock, while, in the other strains, the stressor was without effect. Levels of DA within the nucleus accumbens were not significantly affected by the stressor.

The shock treatment increased DOPAC accumulation within the mesocortex, $F(1,47)=6.44$, $p<0.05$, while the shock treatment × strain interaction did not reach statistical significance. The percentage change of DOPAC in the mesocortex of C3H/HeJ mice, as previously observed (26), was greater than in the other strains, but the small number of mice used, coupled with the marked variability noted in C3H/HeJ mice, precluded a significant strain difference. Likewise, neither the main effect of shock nor the strain × shock interaction involving DA levels approached significance, despite the fact that stressors typically reduce mesocortical DA concentrations (23). In the present investigation, a 14% decline of DA was observed; however, it should be remembered that DA was determined 30 min rather than immediately after stressor termination, which may have afforded the time for DA to return to basal levels.

Pearson product moment correlations were conducted between the NK scores and either the transmitter (NE or DA) or the metabolite (MHPG or DOPAC) levels in stressed and nonstressed mice (30 min following sacrifice). Consistent correlations between NK activity and transmitter or metabolite concentrations in the three strains were not observed, except within the mesocortex. In particular, the correlation between mesocortical DOPAC concentrations and NK activity was .92 and .91 ($n=7$) in nonshocked and shocked BALB/cByJ mice, respectively, .58 and .50 ($n=10$ and 11) in shocked and nonshocked C57BL/6J mice, but only .02 and .35 in the two groups of C3H/HeJ mice. Inasmuch as the significant correlation was noted in both shocked and nonshocked mice of 2 of the 3 strains, it is unlikely that these correlations were spurious.

DISCUSSION

In accordance with earlier reports (6, 12, 28), exposure to an

TABLE 1

MEAN (± S.E.M.) NE, MHPG, DA AND DOPAC CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF NONSHOCKED ANIMALS OF THE SAME STRAIN) IN SEVERAL BRAIN REGIONS OF 3 MOUSE STRAINS

		BALB/cByJ	C57BL/6J	C3H/HeJ
Hypothalamus				
NE	NS	100.00 ± 3.89	99.97 ± 6.30	100.01 ± 6.02
	S	90.36 ± 2.99	82.88 ± 2.85	109.86 ± 3.69
MHPG	NS	99.97 ± 7.37	100.57 ± 14.00	99.99 ± 7.85
	S	145.52 ± 9.33*	166.34 ± 32.62*	192.82 ± 28.69*
Locus Coeruleus				
NE	NS	100.01 ± 16.96	100.01 ± 8.08	99.99 ± 17.20
	S	67.65 ± 8.87*	67.30 ± 3.79*	75.94 ± 11.85*
MHPG	NS	100.00 ± 11.08	100.01 ± 21.31	100.11 ± 18.49
	S	124.01 ± 12.14*	163.07 ± 41.21*	196.86 ± 23.49*
Hippocampus				
NE	NS	100.04 ± 7.74	100.00 ± 7.33	100.07 ± 11.42
	S	59.22 ± 5.57*	81.74 ± 8.65	107.46 ± 17.00
MHPG	NS	100.06 ± 15.34	100.07 ± 11.59	100.01 ± 24.29
	S	145.19 ± 23.28*	147.93 ± 29.73*	97.66 ± 15.87
Mesocortex				
NE	NS	100.04 ± 17.48	100.00 ± 15.30	100.00 ± 15.57
	S	78.28 ± 10.18	75.89 ± 9.41	98.26 ± 12.81
MHPG	NS	99.99 ± 10.96	100.00 ± 23.00	99.98 ± 18.11
	S	153.46 ± 21.28*	186.64 ± 33.72*	135.17 ± 35.23*
DA	NS	99.98 ± 13.48	99.98 ± 15.08	100.00 ± 18.45
	S	75.23 ± 15.73	91.35 ± 12.33	90.87 ± 48.64
DOPAC	NS	100.02 ± 13.14	100.16 ± 8.26	100.03 ± 19.78
	S	139.89 ± 15.20*	102.89 ± 14.21	171.68 ± 42.62*
N. Accumbens				
DA	NS	99.97 ± 10.25	100.00 ± 6.88	100.00 ± 10.06
	S	100.92 ± 11.87	74.74 ± 15.55	94.84 ± 22.94
DOPAC	NS	99.99 ± 8.92	100.00 ± 13.45	99.98 ± 13.18
	S	154.16 ± 14.27*	128.64 ± 11.81	88.04 ± 14.77

*$p<0.05$ relative to nonshocked mice.

Mice were either exposed to 360 Shocks (S) or not shocked (NS). Brain tissue was taken 0.5 hr following stressor termination.

uncontrollable stressor provoked a marked reduction of NK cytotoxicity. Although the NK activity was assessed independently in the three strains, it appeared that the maximal NK reduction induced by the stressor was comparable in the three strains of mice; however, different time-dependent effects of the stressors were evident in the three strains. In C57BL/6J mice, the reduced NK activity was evident within 30 min of stressor termination and was just as marked at the 48-h interval. In C3H/HeJ mice, the appearance of the reduced cytotoxicity was delayed but developed by 24 h and was still evident 48 h after stressor application. Finally, the stressor seemed to have lesser effects in BALB/cByJ mice in that maximal cytotoxicity was not evident until 24 h after stressor termination and recovered to control levels within 48 h of stressor termination. Assuming that NK cytotoxicity might contribute to metastatic disease and in some responses to viral infection (11), the present data might suggest that the effectiveness of stressors in favoring the development of such disorders may not only vary across strains, but also with time following stressor application.

The mechanisms responsible for the stressor-provoked reduction of NK cytotoxicity have yet to be determined, and it is not known what factors might be responsible for the strain-dependent variations in the time course for the effect. It appears unlikely that adrenal corticosteroids were responsible for the stressor-provoked suppression of NK cell cytotoxicity. Using stressor parameters similar to those employed in the present study (360 shocks of 2-s duration, 300 μA), we observed that corticosterone levels were increased in all strains immediately following stressor application (27). Indeed, in C57BL/6J mice, which exhibited the greatest reduction of NK activity, the corticoid increase was less pronounced than in BALB/cByJ mice. Within 30 min of stressor termination, corticoid levels had returned to control values in C3H/HeJ and C57BL/6J mice and were somewhat increased in BALB/cByJ mice. Within 3 h of stressor termination, corticosterone concentrations were at control values in all strains. Thus there does not appear to be any correspondence between the effects of a stressor on NK cell activity and plasma corticosterone concentrations.

The findings of the present investigation concerning the central amine variations induced by stressors are consistent with earlier data showing that stressors will differentially influence NE and DA activity across strains of mice and across brain regions (3,26). Of particular interest with respect to the present investigation was the finding that correspondence between transmitter/metabolite concentrations and the alterations of NK cell cytotoxicity was limited to the mesocortical DA and DOPAC

levels. Mesocortical DA activity is particularly responsive to stressors (23,32), and thus the correlations between mesocortical DA and NK activity may be a reflection of the interindividual or interstrain variability in responsivity to a stressor.

Given that hypothalamic lesions influence immune functioning (5,30), and conversely antigen administration may affect hypothalamic NE activity (2,4), there has been reason to suppose that hypothalamic mechanisms and immune functioning might be mutually regulatory. The fact that there was no correspondence between NK cytotoxicity and hypothalamic NE or MHPG suggests that this component of the immune system is independent of hypothalamic NE activity. Yet it was observed that the stressor-induced NK alterations produced 30 min after stressor exposure in C3H/HeJ were less marked than in the other strains, and that hypothalamic NE levels were least affected by the stressor in this strain. It might thus be tempting to speculate that this would, in fact, suggest correspondence between NE activity and NK cell cytotoxicity. However, analyses of the MHPG accumulation suggests that the utilization of NE in C3H/HeJ mice was as great as in the other strains. Furthermore, the limited amine alterations associated with the stressor in C3H/HeJ were not restricted to the hypothalamus, but were observed in several brain regions. Finally, it should be emphasized that the limited reduction of NK cytotoxicity seen in C3H/HeJ was only apparent 30 min after stressor exposure. At other intervals, the NK cytotoxicity was affected by the stressor, just as in the remaining strains. Thus, in proposing central mechanisms that may be operative in accounting for the variations of NK activity, it may be important to consider not only strain differences that exist, but also the temporal variations in the NK activity which occur in the different strains.

The limited relationships observed between central NE and DA activity and the NK cytotoxicity should not be misconstrued as implying independence of central neurochemical activity and immune functioning. Among other things, the catecholamine analyses in the present investigation were restricted to a few brain regions, and, in some instances, the large tissue samples assayed (e.g., whole hypothalamus as opposed to individual nuclei within this structure) may have obscured potential relationships. Additionally, the accumulation of metabolites was not necessarily reflective of transmitter utilization, but may have reflected, in part, alterations in catabolism or the disposition of catabolic products. Moreover, the proposition cannot be excluded that stressor-induced NE and DA variations contribute to other aspects of immune functioning, such as the T- or B-lymphocyte proliferative response. Finally, the possibility ought to be considered that these transmitters may operate with cotransmitters or interact with other transmitters or hormones in affecting immune functioning. For instance, considerable data have accumulated suggesting that catecholamine variations induced by stressors may be secondary to alterations in opiate peptide activity (1, 14, 32) which may, in turn, act to modulate immune functioning (1,7).

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REFERENCES

1. Anisman, H.; Zalcman, S.; Shanks, N.; Zacharko, R. M. Multisystem regulation of performance deficits induced by stressors: An animal model of depression. In: Animal models of psychiatry. Clifton, N.J.: Humana Press; 1991:in press.
2. Besedovsky, H.; Del Rey, A.; Sorkin, E.; Da Prada, M.; Burri, R.; Honegger, C. The immune response evokes changes in brain noradrenergic neurons. Science 221:564–566; 1983.
3. Cabib, S.; Kempf, E.; Schleef, C.; Oliverio, A.; Puglisi-Allegras, S. Effects of immobilization stress on dopamine and its metabolites in different brain areas of the mouse: Role of genotype and stress duration. Brain Res. 441:153–160; 1988.
4. Carlson, S. L.; Felten, D. L.; Livnat, S.; Felten, S. Y. Alterations of monoamines in specific central autonomic nuclei following immunization in mice. Brain Behav. Immunol. 1:52–63; 1987.
5. Cross, R. J.; Markesbery, W. R.; Brooks, W. H.; Roszman, T. L. Hypothalamic immune interactions: Neuromodulation of natural killer cell activity by lesioning of the anterior hypothalamus. Immunology 51:399–405; 1984.
6. Cunnick, J. E.; Lysle, D. T.; Armfield, A.; Rabin, B. Shock-induced modulation of lymphocyte responsiveness and natural killer activity: Differential mechanisms of induction. Brain Behav. Immun. 2:102–113; 1988.
7. Dantzer, R.; Kelley, K. W. Stress and immunity: An integrated view of relationships between the brain and the immune system. Life Sci. 44:1995–2008; 1989.
8. Esterling, B.; Rabin, B. S. Stress-induced alteration of T lymphocyte subsets and humoral immunity in mice. Behav. Neurosci. 101: 115–119; 1987.
9. Felten, D. L.; Felten, S. Y.; Bellinger, D. L.; Carlson, S. L.; Ackerman, K. D.; Madden, K. S.; Olschowski, J. A.; Livnat, S. Noradrenergic sympathetic neural interactions with the immune system: Structure and function. Immunol. Rev. 100:225–260; 1987.
10. Friedman, S. B.; Glasgow, L. A.; Ader, R. Differential susceptibility to a viral agent in mice housed alone or in groups. Psychosom. Med. 32:285–299; 1970.
11. Herberman, R. B.; Ortaldo, J. R. Natural killer cells: Their role in defenses against disease. Science 214:24–30; 1981.
12. Irwin, M. R.; Hauger, R. L. Adaptation to chronic stress: Temporal pattern of immune and neuroendocrine correlates. Neuropsychopharmacology 1:239–242; 1988.
13. Irwin, M.; Vale, W.; Rivier, C. Central corticotropin-releasing factor mediated the suppressive effects of stress on natural killer cell cytotoxicity. Endocrinology 126:2837–2844; 1990.
14. Kalivas, P. W.; Widerlov, E.; Stanley, D.; Breese, G. R.; Prange, A. J. Enkephalin action on the mesolimbic dopamine system: Dopamine-dependent and dopamine-independent increase in locomotor activity. J. Pharmacol. Exp. Ther. 16:229–237; 1983.
15. Laudenslager, M. L.; Ryan, S. M.; Drugan, R. C.; Hyson, R. L.; Maier, S. F. Coping and immunosuppression: Inescapable but not escapable shock suppresses lymphocyte proliferation. Science 221: 568–570; 1983.
16. Livnat, S.; Felten, S. Y.; Carlson, S. L.; Bellinger, D. L.; Felten, D. L. Involvement of peripheral and central catecholamine systems in neural-immune interactions. J. Neuroimmunol. 10:5–30; 1985.
17. Livnat, S.; Irwin, J.; Ader, R.; Gallo, K. L.; Anisman, H. Immunological reactivity following acute and chronic stress. Soc. Neurosci. Abstr. 11:861; 1985.
18. 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.
19. Okimura, T.; Nigo, Y. Stress and immune responses I: Suppression of T cell function in restraint-stressed mice. Jpn. J. Pharmacol. 40: 505–511; 1986.
20. Pross, H. F.; Baines, M. G.; Rubin, P.; Shragge, P.; Patterson, M. S. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. J. Clin. Immunol. 1:51–63; 1981.
21. Rabin, B. S.; Lyte, M.; Hamill, E. The influence of mouse strain and housing on the immune response. J. Neuroimmunol. 17:11–16; 1987.
22. Rossier, J.; Guillemin, R.; Bloom, F. Footshock induced stress decreases leu^5-enkephalin immunoreactivity in rat hypothalamus. Eur.

J. Pharmacol. 48:465–466; 1978.
23. Roth, R. H.; Tam, S-Y.; Ida, Y.; Yang, J. X.; Deutsch, Y. Stress and the mesocorticolimbic dopamine systems. Ann. NY Acad. Sci. 537:138–147; 1988.
24. Seegal, R. F.; Brosch, K. O.; Bush, G. High-performance liquid chromatography of biogenic amines and metabolites in brain, cerebrospinal fluid, urine and plasma. J. Chromatogr. 377:131–144; 1986.
25. Shanks, N.; Anisman, H. Stressor-provoked behavioral changes in six strains of mice. Behav. Neurosci. 102:894–905; 1988.
26. Shanks, N.; Zalcman, S.; Zacharko, R. M.; Anisman, H. Alterations of central norepinephrine, dopamine and serotonin in several strains of mice following acute stressor exposure. Pharmacol. Biochem. Behav. 38:69–75; 1991.
27. Shanks, N.; Griffiths, J.; Zalcman, S.; Zacharko, R. M.; Anisman, H. Mouse strain differences in plasma corticosterone following uncontrollable footshock. Pharmacol. Biochem. Behav. 36:515–519; 1990.
28. Shavit, Y.; Lewis, J. W.; Terman, G. W.; Gale, R. P.; Liebskind, J. C. Opioid peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity. Science 233:188–190; 1984.
29. Shavit, Y.; Martin, F. C.; Yirmiya, R.; Ben-Eliyahu, S.; Terman, G. W.; Weiner, H.; Gale, R. P.; Liebeskind, J. C. Effects of a single administration of morphine or footshock stress on natural killer cell cytotoxicity. Brain Behav. Immun. 1:318–328; 1987.
30. Stein, M.; Schleifer, S. J.; Keller, S. E. Hypothalamic influences on immune responses. In: Psychoneuroimmunology. New York: Academic Press; 1981:429–47.
31. Weiss, J. M.; Sundar, S. K.; Becker, K. J.; Cierpial, M. A. Behavioral and neural influences on cellular immune responses: Effects of stress and interleukin-1. J. Clin. Psychiatry 50:43–55; 1989.
32. Zacharko, R. M.; Anisman, H. Pharmacological, biochemical and behavioral analyses of depression: Animal models. In: Animal models of depression. Boston: Birkhauser; 1989:204–238.
33. Zalcman, S.; Minkiewicz-Janda, A.; Richter; Anisman, H. Critical periods associated with stressor effects on antibody titers and on the plaque forming cell response to sheep red blood cells. Brain Behav. Immunol. 2:254–266; 1988.
34. Zalcman, S.; Richter, M.; Anisman, H. Alterations of immune functioning following exposure to stressor-related cues. Brain Behav. Immunol. 3:99–109; 1989.