REFERENCES

1. A. F. Blyuger, *Klin. Med.*, No. 2, 51-59 (1987).
2. Kh. Ya. Karimov, in: *Topics of Current Interest in Medicine* [in Russian], Tashkent (1993), pp. 3-8.
3. Kh. Ya. Karimov, B. U. Iriskulov, and M. K. Ergashev, *Byull. Eksp. Biol. Med.*, **116**, No. 12, 584-585 (1993).
4. V. I. Udovichenko, *Pat. Fiziol*, No. 1, 73-75 (1978).
5. D. E. McMillan, *Microcirc. Endothelium Lymphatics*, **1**, No. 1, 3-34 (1984).
6. B. G. Stone and D. H. Van-Thiel, *Semin. Liver Dis.*, **5**, No. 1, 8-28 (1985).

Prevention of Immunosuppression in Stressed Mice by Altering the Activity of Neurotransmitter Systems

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The spleens of CBA mice stressed by being immobilized for 3 h in the supine position and then immunized with sheep erythrocytes showed evidence of immunosuppression manifested in reduced numbers of plaque-forming cells on day 4 and of rosette-forming cells on day 5 after the stress and immunization. The depletion of serotonin stores in the brain caused by p-chlorophenylalanine administered 48 h before stressing the animals abolished immunosuppression under the action of immobilization stress, and a similar effect resulted from the activation of postsynaptic dopamine receptors D_1 and D_2 by apomorphine injected at 30 min before stress. The prevention of immunosuppression observed to occur when the balance between the serotoninergic and dopaminergic systems was shifted so that the latter system became predominant, suggests that the stress reduces immune reactivity by altering the brain's neurochemical pattern and interfering with the mechanisms of neuroimmunomodulation.

Key Words: *stress; immunosuppression; serotoninergic and dopaminergic systems*

It is evident from what is currently known about relationships between the brain, psyche, and endocrine and immune systems [3,6,10,11] that impairment of the mechanisms underlying neuroimmunomodulation may result in altered immune reactivity. Immunological functions can be compromised by stressors of different kinds [8,15]. Although the neurochemical pattern of the brain after stressful exposure varies with the nature of stressor [1], serotonin synthesis and the activity of serotoninergic neurons increase in response to a number of stressors, including immobilization [8,9,12,13], while the dopamine level then remains unchanged or declines [4], but the rate of dopamine metabolism may rise in some brain structures [5]. Studies carried out at our institute have provided information on the importance for immune response development of a balance between the serononinergic (immunosuppressing) and dopaminergic (immunostimulating) systems at the time when antigen enters the body [3,11]. We showed also that a pharmacologically elicited elevation of the dopaminergic system's activity in stressed old C57Bl/6 mice can raise their immune response to the level characteristic of unstressed young animals [2]. Altering the brain's neurochemical pattern by pharmacological means may therefore be expected to open new avenues for preventing stress-induced immunosuppression.

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MATERIALS AND METHODS

A total of 113 male CBA mice aged 2.0-2.5 months was used, divided into six groups as follows: control mice given only physiological saline (group I); stressed mice immunized immediately after the stress (group II, which also received saline); mice given the tryptophan hydroxylase blocker p-chlorophenylalanine (p-CPA; Calbiochem-Behring Corp.) at 500 mg/kg 48 h before immunization (group III); mice given p-CPA in the indicated dose 48 h before being stressed and immunized immediately thereafter (group IV); mice given apomorphine (1 mg/kg), an agonist of postsynaptic dopamine receptors D_1 and D_2, 3.5 h before immunization (group V); mice given apomorphine at 1 mg/kg 30 min before stress (3.5 h before immunization) (group VI). Animals were stressed by being immobilized on the back with rubber bandages for 3 h (from 09:00 until 12:00 h), and they were immunized with 5×10^8 sheep erythrocytes. There were no less than 10 mice per group.

The immune response was evaluated at its peak in the spleen of mice by counting plaque-forming cells (PFC) on day 4 and rosette-forming cells (RFC) on day 5 after immunization [11].

The effects from the stress and the compounds used were estimated by Student's *t* test.

RESULTS

The three-hour immobilization of mice in the supine position resulted in a strong inhibition of the immune response. Thus, the number of PFC per 10^6 cells as well as their number per spleen were decreased by more than a half as compared to the unstressed controls, and the spleen weight also decreased (Table 1: gr. II *vs.* gr. I), as did, almost by a factor of IV, the number of RFC per 10^3 cells (Table 2).

p-CPA in high doses produces a long-lasting depression of serotonin levels in the brain, which reached its maximum on day 2 postadministration [14]. Elevation of serotonin synthesis in stressed animals was prevented by preinjecting them with p-CPA [13]. In our study, no immunosuppression was noted in group IV injected with p-CPA at 500 mg/kg 48 h before the immobilization stress: PFC counts per 10^6 cells and per spleen in this group did not differ significantly from those in the p-CPA-treated unstressed group III and were approximately two times as high as in the p-CPA-untreated stressed group II (Table 1), while RFC counts were significantly higher than in the control group and in group III (Table 2); group IV did not differ from the control group in spleen weight (Table 1). In contrast to the findings of our study, p-CPA treatment in small doses during a four-day period of chronic stress caused no change in the immune response in comparison with untreated stressed animals [8]. The effect of p-CPA on the immune response probably depends on its doses and the time of its administration in relation to the day of immunization.

p-CPA was shown previously to stimulate the immune response via the dopaminergic system: when the postsynaptic dopaminergic receptors were blocked by haloperidol, no stimulation was observed [3].

In animals stressed by immobilization, the activity of the serotoninergic system is, as noted above, elevated, whereas that of the dopaminergic system remains unchanged or is lowered [4,9]. Significant falls of dopamine were recorded in brain areas A_I, A_{IX}, and A_X (by 56% in A_{IX}) in rats exposed to immobilization stress for 6.5 h [4]. Since the nigrostriatal dopaminergic system participates in neuroimmunomodulation [10], the recorded immunodepression could be associated with reduced activity of the dopaminergic system. We observed normalization of the immune response upon activation of this system in stressed old C57Bl/6 mice [2] having lowered dopamine levels and reduced densities of D_1 and D_2 dopaminergic receptors. In the present study, the postsynaptic dopamine receptor apomorphine injected into young CBA mice at 1 mg/kg [7] before

TABLE 1. Effects of Stress and p-Chlorophenylalanine (p-CPA) on the Plaque-Forming Cell (PFC) Count and Spleen Weight in CBA Mice on Day 4 after Stress and Immunization with Sheep Erythrocytes (5×10^8) ($M\pm m$; n=10 in each group)

Parameter	Groups			
	I control	II stress	III p-CPA	IV p-CPA+stress
PFC count/10^6 cells	56.6±20.8	140.2±4.1*	350.7±9.3*	255.1±18.3**
PFC count/spleen	23505±1151	10073.8±436.3*	33115.6±1172.8*	22851.9±1462.8**
Spleen weight, mg	92±3.6	71.8±2.3*	94.2±2.4	90.7±4.6**

Note. Here and in Table 2: $p<0.001$: *in comparison with group I; **in comparison with group II.

TABLE 2. Effects of p-Chlorophenylalanine (p-CPA) and Apomorphine on the Immune Response in Stressed CBA Mice on Day 5 after Immunization with Sheep Erythrocytes (5×10^8) (*M±m*)

RFC count per 10^3 cells in the six groups					
I control	II stress	III p-CPA	IV p-CPA+stress	V apomorphine	VI apomorphine+stress
18.7±1.3 (15)	8.5±0.6* (18)	32.7±2.3* (10)	22.0±0.3*,** (10)	6.5±1.1* (10)	18.5±0.7** (10)

Note. Figures in parentheses are the number of animals.

stress was found to abolish its immunosuppressing action; the RFC count in this group VI) was almost the same as in the control group (Table 2).

The results of this study, together with the previously reported information on the role of neurotransmitter systems and on their interaction in the process of neuroimmunomodulation [3,10], suggest that stress decreases immunological reactions by altering the brain's neurochemical pattern dominated by the serotoninergic system. When this system is depleted or dopamine receptors are activated, the balance between the serotoninergic and dopaminergic systems shifts so that the latter system, which, as we showed earlier [10], is immunostimulating, comes to predominate. Pharmacological agents targeting particular neurochemical systems of the brain may bring about reconstitution of the normal immune status.

REFERENCES

1. A. V. Gorbunova and E. I. Belova, *Zh. Vyssh. Nervn. Deyat.*, **42**, No. 2, 363-371 (1992).
2. L. V. Devoino, G. V. Idova, and M. A. Cheido, *Byull. Eksp. Biol. Med.*, **116**, No. 11, 532-534 (1993).
3. L. V. Devoino and R. Yu. Ul'yuchenok, *Monoaminergic Systems in the Regulation of Immune Reactions (Serotonin, Dopamine)* [in Russian], Novosibirsk (1983).
4. T. M. Ivanova, R. Kventanskii, T. I. Belova, *et al.*, *Fiziol. Zh. SSSR*, **71**, No. 7, 823-828 (1985).
5. E. D. Abercrombie, K. A. Keefe, D. S. DiFrishia, *et al.*, *J. Neurochem.*, **52**, 1655-1658 (1989).
6. R. Ader, D. Felten, and N. Cohen, *Ann. Rev. Pharmacol. Toxicol.*, **50**, 561-602 (1990).
7. S. Arunasmitha, C. Andrade, and N. Pradhan, *Indian J. Physiol. Pharmacol.*, **33**, No. 2, 132-136 (1989).
8. M. Boranic, D. Pericic, M. Poljaak-Brasi, *et al.*, *Ann. N. Y. Acad. Sci.*, **496**, 485-491 (1987).
9. F. Crespi, *Regul. Pept.*, **26**, No. 1, 65-68 (1989).
10. L. Devoino, E. Alperina, and G. Idova, *Int. J. Neurosci.*, **40**, No. 3, 271-288 (1988).
11. L. Devoino, G. Idova, E. Alperina, and M. Cheido, *Brain Res.*, **30**, 267-274 (1994).
12. A. J. Dunn, *Int. J. Neurosci.*, **63**, No. 1-2, 45-46 (1992).
13. M. H. Joseph and G. A. Kennet, in: *Progress in Serotonin Research*, New York (1984), pp. 383-386.
14. F. Richard, J. L. Sanne, O. Bourde, *et al.*, *Brain Res.*, **536**, 41-45 (1990).
15. T. M. Wolf, B. Cole, S. Fahrion, *et al.*, *Int. J. Neurosci.*, **79**, 121-132 (1994).