Suppression of the immune response by drugs interfering with the metabolism of serotonin

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Summary. The work was based on the assumption that neurohumoral control of the immune response, particularly in stressed animals, involves central serotoninergic mechanisms. Rats immunized with sheep erythrocytes were stressed by repeated restraints and/or treated with a precursor of serotonin (5-hydroxytryptophan, 5-HTP) or with an inhibitor of serotonin synthesis (parachlorophenylalanine, PCPA). As expected, repeated stresses reduced the plaque-forming cell (PFC) response. Treatment with 5-HTP also reduced the PFC response, and potentiated the immunosuppressive effect of stress. This was accompanied by increased metabolism of serotonin in the brain, as indicated by increased concentration of its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in cerebral tissue. Treatment with PCPA also suppressed the PFC response, but this suppression was accompanied by decreased levels of brain serotonin and of 5-HIAA. Plasma corticosterone levels were elevated in rats treated with PCPA. It seems that putative central effects of PCPA on serotoninergic regulation of the immune response were outweighed by its effects on corticosterone secretion and/or on lymphoid cells.

Key words. Rat plasma; plasma, rat; immune response; stress; immunosuppressive drugs; serotonin, brain; corticosterone, plasma; plaque-forming cell response.

Suppression of the immune response by prolonged or repeated stress has been associated, in our experiments, with increased metabolism of serotonin in the brains of rats[1]. This was in agreement with ideas of Devoino et al.[2] and other authors[3] that central serotoninergic pathways, especially in the hypothalamus, play an important role in the control of the immune response at the level of whole organism. If so, alterations of the metabolism of serotonin might be able to modify the immune response, particularly in stressed animals. We chose 2 drugs expected to exert opposite effects: 5-hydroxytryptophan (5-HTP) – the immediate precursor of serotonin, and parachlorophenylalanine (PCPA) – an inhibitor of serotonin synthesis. Their effects were compared in nonstressed rats immunized with sheep erythrocytes, and in immunized rats stressed by repeated restraints. The plaque-forming cell (PFC) response, the concentrations of monoamines in the brain, and the levels of corticosterone in plasma were measured in these animals.

Materials and methods. Rats. Male Wistar rats, 8–10 weeks old, weighing 170–250 g, were used. They were housed in groups of 5, allowing an adaptation period of 2 weeks before the experiment.

Stress. The animals were restrained on boards by means of adhesive tape on 4 consecutive days for 3 h/day (between 08.00 and 11.00). The first restraint was started 2 h after the injection of antigen. Control (nonstressed) rats were handled in the same laboratory environment, and injected with drugs or solvents.

Drugs. These were given by i.p. injections on 4 consecutive days, starting the first administration 1 h after the injection of antigen. In stressed animals, the drugs were given 1 h before the restraint, i.e. the sequence of the treatments was: antigen – drug – restraint. DL-parachlorophenylalanine (PCPA) (Sigma, St. Louis, MO, USA) was dissolved in 1 N HCl, diluted with distilled water, and the pH was adjusted to 3 by 1 N NaOH. L-5-hydroxytryptophan (5-HTP) (Sigma) was dissolved in saline. The doses were 750 µmol/kg and 390 µmol/kg (150 mg/kg and 100 mg/kg) per day, respectively. Rats without drugs (controls) received injections of both solvents.

Immune response. The rats received i.p. injections of 2×10^9 sheep red blood cells (SRBC), and the numbers of hemolytic plaque-forming cells (PFC) were determined in their spleens by the Jerne technique 5 days later.

Monoamines in brain tissue and corticosterone in plasma. Rats were quickly decapitated by guillotine 24 h after the last injection of drug or saline (i.e. 20 h after the last restraint, if stressed), and the trunk blood was collected in heparinized beakers. Fluorimetric methods[1] were used for assaying serotonin, its metabolite 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline and dopamine in cerebral tissue, and for determination of corticosterone in plasma. 5-HTP does not interfere with this assay.

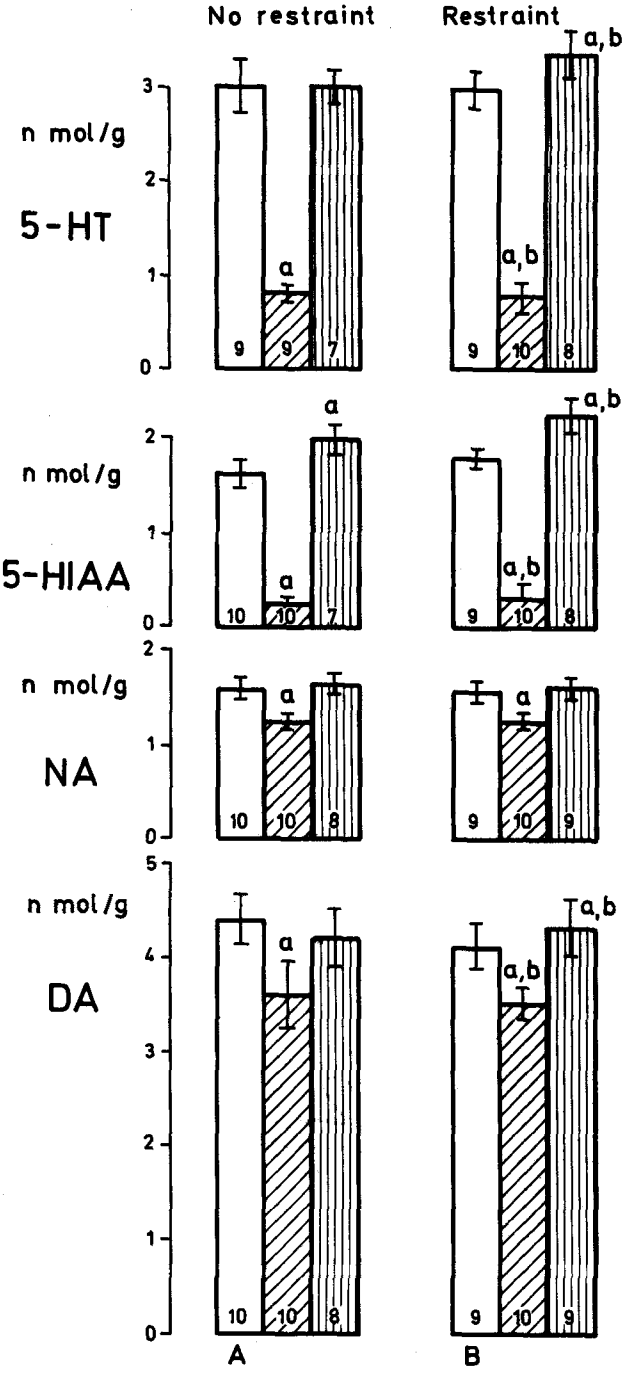
Statistics. Student's *t*-test was employed, and the level of significance was set at 5% ($p < 0.05$ or better).

Results. As expected, treatment with PCPA (inhibitor of serotonin synthesis) sharply decreased the concentrations of serotonin and its metabolite 5-HIAA in the brains of rats. The effect in restrained rats was quite similar (fig.). Treatment with a precursor of serotonin, 5-HTP, increased the levels of 5-HIAA in nonstressed and stressed rats alike. The level of serotonin was significantly increased only in stressed rats. Noradrenaline and dopamine were clearly decreased in the brains of rats treated with PCPA (stressed and nonstressed alike). Treatment with 5-HTP slightly increased the concentration of dopamine in stressed rats. Confirming our previous experience, repeated restraints diminished the PFC response to sheep erythrocytes (table). Treatment with 5-HTP also diminished the immune response to SRBC, and in stressed rats even augmented the inhibitory effect of stress (table). Treatment with PCPA was also immunosuppressive, but no difference between stressed and nonstressed rats was noted. The concentration of 5-HIAA, the metabolite of serotonin, was somewhat increased in the brains of stressed rats, but the increase noted in previous work did not reach statistical significance (fig.). The levels of corticosterone in plasma, measured at the time of sacrifice (i.e. 20 h after the last restraint or 24 h after the last drug treatment) were still slightly (but not significantly) elevated in stressed rats. In rats treated with PCPA (whether stressed or not) the corticosterone levels at the time of sacrifice were evidently high. Slightly increased levels of plasma corticosterone in rats treated with 5-HTP did not reach statistical significance.

Discussion. The results show that 2 drugs exerting opposite effects on the metabolism of serotonin – a precursor (5-HTP) and an inhibitor of serotonin synthesis (PCPA) influenced immune response in the same way: both were immunosuppressive. An immunosuppressive effect of 5-HTP has been shown by Devoino et al.[2] in mice and rabbits. Since disruption of connections between hypothalamus and pituitary abolished this effect, Devoino et al. concluded that brain serotonin, increased by its precursor 5-HTP, interfered with the immune response via neuroendocrine mechanisms. Pierpaoli and Maestroni[4,5] suppressed PFC response in mice by 5-HTP given in combination with phentolamine and haloperidol, and postulated that immunosuppression resulted from central stimulation of serotoninergic receptors together with a block of dopaminergic and α-adrenergic ones.

Literature data about effects of PCPA on the immune response are contradictory. Pierpaoli and Maestroni[5,6] used PCPA in combination with dopamine, adrenergic blockers, and haloperidol, and found some inhibition of PFC response in mice, but

rejection of skin grafts was accelerated. Vinnitsky and Yakimenko[7] observed partial restoration of immune reactivity, suppressed by methylcholanthrene, after treating the mice with PCPA.



Effect of para-chlorophenylalanine (PCPA), 5-hydroxytryptophan (5-HTP), repeated restraints, or combinations thereof, applied on 4 consecutive days, on the levels of serotonin (5-hydroxytryptamine, 5-HT), its metabolite 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA) and dopamine (DA) in the brains of rats, determined on the 5th day i.e. 24 h after the last injection of drug. Pooled results of 2 experiments (the same as in the table). Columns indicate mean values, with standard deviations, and with numbers of animals at the bottoms. Small letters (a, b) indicate significant differences (p 0.05 or better) from the corresponding control groups indicated with capital letters (A, B). ☐ No drug (solvent); ▨ PCPA; ▥ 5-HTP.

We expected PCPA to promote the immune response, and particularly to abrogate the immunosuppressive effect of stress. However, PCPA was evidently immunosuppressive with the treatment schedule employed. Since the brain serotonin level was decreased (as expected) this finding cannot be readily reconciled with views that neuroendocrine inhibition of the immune response was coupled with increased concentration and/or metabolism of serotonin in the brain[1-3,7]. Perhaps some other effects of PCPA outweighed its putative effect on central serotoninergic control of the immune response. Indeed, treatment with PCPA resulted in sustained elevation of plasma corticosterone 1 day after the last injection, while in rats treated with 5-HTP and/or restrained, corticosterone approached resting levels.

Many drugs that enhance serotoninergic neurotransmission elevate plasma corticosterone in rats,[8,9] and this is consistent with views that serotoninergic pathways stimulate the hypothalamo-pituitary-adrenal (HPA) axis[10-13]. Our results – sharp decrease of 5-HT and 5-HIAA in brain together with sustained elevation of plasma corticosterone after treatment with PCPA – recall opposite views, i.e. that the HPA axis was inhibited by serotonin[14-16]. Similar increase of plasma corticosterone with PCPA has been described,[9,17] but correlation with brain levels of 5-HP and 5-HIAA was not seen[20]. Another possibility would be that PCPA affected the HPA axis via noradrenergic neurons. Noradrenaline is known to inhibit the HPA axis[18], and we found (like others[19,20]) decreased levels of brain noradrenaline and dopamine in rats treated with PCPA. Finally, PCPA might have interfered with the immune response by direct effects on lymphoid cells. This question, and also the possibility that 5-HTP (or 5-HT formed from it at the periphery) exerted such effects, are being investigated by appropriate experiments in vitro.

A few qualifications should be added. Since our intention was to see whether repeated stresses, in combination with drugs affecting serotoninergic transmission, would result in gross and sustained alterations of neuroendocrine status affecting the immune response, we have measured monoamine levels in brain and corticosterone in plasma one day after the last restraint, thus allowing the immediate effects of restraint, such as high levels of corticosterone,[21] to abate. For the same reason we assessed monoamines in whole brain, disregarding in this way subtle alterations in discrete areas of the brain[3]. Nevertheless, this crude method still yielded useful information. Finally, our measurements were all done in animals developing an immune reaction. In this situation, neuroendocrine status differs from that in nonimmunized animals, since the immune response is not only controlled by neuroendocrine mechanisms, but also affects them in its turn[5,22,23].

Effect of para-chlorophenylalanine (PCPA), 5-hydroxytryptophan (5-HTP) repeated restraints, or combinations thereof, applied on 4 consecutive days after immunization of rats with sheep erythrocytes, on the PFC counts in the spleens and corticosterone levels in plasma, determined on the 5th day 24 h after the last injection of drug. Pooled results for 2 experiments; in parentheses, numbers of rats (discrepancies are due to lost samples)

Drug injected	No restraint	Restraint
	PFC counts per spleen ($\times 10^3$) (means ± SEM)	
None (solvent only)	513.3 ± 86.2 (8)	224.4 ± 40.5 (9)*
PCPA (750 µmol/kg/day)	211.1 ± 30.4 (10)*	240.2 ± 60.7 (9)*
5-HTP (390 µmol/kg/day)	248.5 ± 85.2 (6)*	114.0 ± 14.4 (7)*° ≠
	Corticosterone in plasma (nmol/l) (means ± SEM)	
None (solvent only)	338.8 ± 38.7 (9)	400.9 ± 49.4 (10)
PCPA (750 µmol/kg/day)	824.8 ± 128.7 (8)*	864.6 ± 46.8 (8)* ≠
5-HTP (390 µmol/kg/day)	460.3 ± 115.4 (10)	525.3 ± 50.2 (10)

Symbols indicate significant differences ($p < 0.05$ or better) from groups treated with: * no drug, no restraint; ° 5-HTP, no restraint; ≠ no drug, restraint.

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Evidence for no relationship of the pineal activity with the neonatal shrinkage of Leydig cells in the rat[1]

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Summary. The collective volume of Leydig cells in prenatally pinealectomized newborn rats declined as sharply as in intact newborn rats. Also, the collective volume in pups born in the dark declined in a fashion similar to that in pups born in the light. The results indicate that neither the pineal gland nor the light is responsible for the neonatal shrinkage of Leydig cells.
Key words. Leydig cell; pineal gland; neonate; fetus, rat.

The drastic neonatal decline of the collective volume of Leydig cells has been well documented[3,4]. This decline is neither due to the disappearence of transplacental maternal LH nor to the cessation of supply of chorionic gonadotrophin (CG). Maternal pituitary LH cannot cross the placenta[5]. It has been recently shown that rat placental extract contains a trace amount of CG[6]. However, the activity of CG during fetal stages seems to be too low to maintain the growth of Leydig cells, since fetal hypophysectomy stops the growth of Leydig cells[3,7,8]. Also, the cessation of supply of maternal estrogen does not seem to be responsible for the neonatal shrinkage of Leydig cells[9].

It must be considered that the pineal gland of the newborn rat may act as a factor in the shrinkage of Leydig cells, since the pineal glands of fetal rats near term as well as of newborn rats can release some melatonin in organ culture[10]. It is well established that melatonin inhibits the output of LH by influencing the hypothalamic-pituitary system[11,12], and that the synthesis of melatonin in the pineal gland and its release occur with a diurnal rhythmic high level at night and a low level during the day[13].

In general, birth in the rat occurs during the day, not at night. It is quite possible, therefore, that the light at birth activates the pineal gland in some way, and this then produces melatonin during the 1st night following the birth. On the basis of this view, it is possible that in pups born and maintained in the dark, the pineal gland is not activated for melatonin production. The present work was designed to test these possibilities by observation of changes in the collective volume of Leydig cells following prenatal pinealectomy or following spontaneous birth in the dark.

Materials and methods. Wistar rats were fed a commercial diet (Labo MR Breeder) and water. The morning on which mating was detected by the presence of sperm in the vaginal smear was regarded as day 1 of gestation. In our rat colony spontaneous delivery occurred around noon on day 22 of gestation. The next day was counted as day 1 after birth.

In the 1st series of experiments, 6 pregnant rats were subjected to a midventral laporotomy under ether anesthesia on day 20 of gestation. Then 2 male fetuses in each litter were subjected to pinealectomy. The sex was determined by viewing the fetus through the translucent uterine wall; the distance between the genital tubercle and the anus was clearly longer in the male than in the female. For fetal pinealectomy, a fine tapered glass tube connected with vinyl tubing to a water aspirator was used. The tube was inserted through the uterine wall so as to pierce the fetal skull at a point on the sagittal suture just behind the cross of the coronal-sagittal sutures, and the fetal pineal gland was removed by aspiration. Two other male fetuses in the same litter were sham-operated. Autopsy was performed on day 22 of gestation or day 1 after birth. At autopsy, these pinealectomized and sham-operated animals were matched at random with 2 intact littermates.

In the 2nd series of experiments, 3 pregnant rats were housed individually on day 20 of gestation in a dark room and were delivered of their pups in the dark. Delivery in the dark was usually delayed. The pups born by 20.00 h on day 22 of gestation were used in this study, and the pups born later were dis-