Relative Resistance of LEW Rats to Rotation Stress

by *Bjarne Klausen,* Royal Dental College, Nørre Allé 20, DK-2200 Copenhagen N, Denmark & *Hans Petter Hougen,* Departments of Oral Diagnosis, Microbiology and Periodontology, Royal Dental College; Bertholin Institute, Kommunehospitalet; Copenhagen, Denmark.

Introduction

Psychological stress in generally considered an important contributory factor in the development of various diseases (Sklar & Anisman, 1979; Brodsky *et al.,* 1987; Levy *et al.,* 1987; Stoney *et al.,* 1987). In particular, the state of mind seems to have profound influence on the immune system (Riley, 1981; Bovbjerg *et al.,* 1982; Besedovsky *et al.,* 1983; Landenslager *et al.,* 1983; Glaser & Kiecolt-Glaser, 1986; Ghoneum *et al.,* 1987).

Therefore, in experimental pathology studies, stress methods that are applicable to laboratory animals are desirable. However, the borderline between stress and torture is difficult to draw, i.e. it may be a problem to find methods that are efficient, but not traumatic to the animals.

Riley (1981) has described body rotation as a »simple, reproducible, and nontraumatic means for the quantitative induction of simple anxiety stress in experimental animals«. In mice, body rotation is associated with an increased plasma corticosterone level, dramatic reductions in blood leukocyte counts and thymus weight, as well as decreased ability to control tumour growth (Riley, 1981).

In rats, exposure to body rotation has been shown to cause reductions in spontaneous activity (Erskin & Riccio, 1966), increase in defaecation responses (Ossenkopp & Frisken, 1982), and suppression of drinking (Haroutunian *et al.,* 1976); but to our knowledge effects on immunological parameters have not been examined.

The purpose of the present study was to test the effect of body rotation on the weight of lymphoid organs, and cellularity of peripheral blood and lymph in rats.

Animals

A total of 30 LEW/Mol rats (12 female, 18 male) were used. The animals were 5 weeks old when the experiments started. The rats were kept under SPF conditions in Makrolon type III cages with stainless steel tops (Scanbur Ltd., Køge, Denmark) and sterile cage bedding. They had Altromin 1410 food pellets (Altromin International Tier-Labor-Service, Lage, FRG) and distilled drinking water *ad libitum.*

Experimental design

The rats were rotated by placing their cages on the turntable of a grammophone for 10 min at 78 revolutions per minute (rpm). All animals had five daily rotation periods with 50 min break between. In one experiment 5 rats (4 male, 1 female) were rotated for four days, whereas 5 rats (3 male, 2 female) served as controls. In another experiment 9 rats (5 male, 4 female) were rotated for twenty days, and the control group consisted of 11 rats (6 male, 5 female). All animals were killed by cervical dislocation 24 h after the last rotation period. Before killing the rats were anaesthetized with Brietal (Lilly, Indianapolis, IN, USA) 0.01 ml of a 2% solution per gram body weight. Lymph was obtained from a cervical thoracic duct fistula. Blood was obtained from the right subclavian artery, the animals were exsanguinated and organs were weighed. Cells from thoracic duct lymph were diluted in Türk's solution and counted in Bürker Türk counting chambers. Peripheral blood leukocyte numbers were counted in a Coulter Counter Model 5s (Coulter Electronics Ltd., Harpenden, Herts, UK). In the second experiment, inguinal lymph nodes were fixed in formalin, embedded in paraf-

fin wax, cut in 5 μm sections and stained with haematoxylin and eosin for histological examination. During histological examination the number of lymphocytes in 40.000 μm^2 of the paracortex was counted at 500 × magnification.

Statistical analyses

All values were expressed as arithmetic means ± standard error of the mean. Differences between stressed and control animals were tested by the Mann-Whitney U test. For the rejection of hypotheses the 5% level of significance was chosen.

Results

During rotation periods the rats moved to the periphery of the cage, and there they resumed their previous activities.

The results of the short time (4 × 5 × 10 × 78 rpm) experiment are shown in Table 1. It is seen that the means of all examined variables were lower in stressed animals than in unstressed controls. However, the difference was only significant with respect to spleen weight ($p = 0.037$), whereas the difference in thymus weight was close to the 5% level of significance ($p = 0.060$). However, when organ weights were corrected for differences in body weight, thymus weight and spleen weight appeared similar in rotated and control rats.

In the long time (20 × 5 × 10 × 78 rpm) experiment (Table 2), similar tendencies appeared: All variables (except corrected spleen and thymus weights) were lower in stressed rats. But in this case, none of the differences were significant.

In pilot studies at 45 rpm for less than four

Table 1. Effects of rotation stress for four days.

	Rotated rats (n = 5)	Control rats (n = 5)	Statistics
Weight gain	27.8 ± 11.8	55.8 ± 8.3	p = 0.095
Spleen weight	466.8 ± 38.6	581.5 ± 17.5	p = 0.037
Corrected spleen weight	329.6 ± 21.5	331.3 ± 26.4	p > 0.1
Thymus weight	225.9 ± 35.3	318.0 ± 44.2	p = 0.060
Corrected thymus weight	155.6 ± 11.1	183.1 ± 35.4	p > 0.1
Inguinal lymph node weight	5.6 ± 1.0	6.7 ± 1.6	p > 0.1
Corrected lymph node weight	4.1 ± 0.8	3.7 ± 0.3	p > 0.1
Thoracic duct lymphocyte number	25.2 ± 5.2	43.5 ± 7.0	p > 0.1
Peripheral blood leukocyte number	5.7 ± 1.1	6.0 ± 0.8	p > 0.1

All values are the mean ± the standard error of the mean. Weight gain is in grams, organ weights in mg, corrected organ weights in mg pr. 100 g body weight, cell numbers are x 10^6 ml^{-1}.

Table 2. Effects of rotation stress for twenty days.

	Rotated rats (n = 9)	Control rats (n = 11)	Statistics
Weight gain	103.0 ± 10.1	122.8 ± 10.3	p = 0.094
Spleen weight	581.8 ± 23.5	609.0 ± 27.1	p > 0.1
Corrected spleen weight	277.5 ± 11.3	273.8 ± 6.1	p > 0.1
Thymus weight	296.9 ± 24.9	308.9 ± 33.8	p > 0.1
Corrected thymus weight	141.4 ± 11.9	139.8 ± 15.4	p > 0.1
Inguinal lymph node weight	9.4 ± 0.7	15.1 ± 2.4	p > 0.1
Corrected lymph node weight	4.5 ± 0.4	6.6 ± 0.8	p = 0.068
Thoracic duct lymphocyte number	35.8 ± 4.4	44.9 ± 3.9	p > 0.1
Peripheral blood leukocyte number	2.3 ± 0.6	2.8 ± 0.2	p > 0.1
Paracortical lymphocyte number	1286.5 ± 107.9	1479.8 ± 91.8	p = 0.092

Paracortical lymphocytes are cells per 40.000 μm^2, other values: see Table 1.

days, we found no differences at all between stressed and unstressed rats (data not presented).

Discussion

The effects of body rotation found in the present study differ considerably from results previously described in mice. In mice, 40-50% reductions in peripheral blood leukocyte (PBL) number and thymus weight were seen after rotation for one day at 45 rpm (Riley, 1981). In rats, we found no effects at 45 rpm, and after four days at 78 rpm only minor and insignificant reductions were seen in PBL, whereas thymus weight was reduced moderately. After four days insignificant reductions were also found in body weight gain, inguinal lymph node weight and thoracic duct lymphocyte number, whereas a significant decrease was seen in spleen weight. The differences in lymphoid organ weights probably are mere reflections of differences in body weight; but the reduced PBL and thoracic duct lymphocyte number in rotated rats do suggest that these animals had a slight generalized lymphopenia. Furthermore it appeared that body rotation somehow interfered with the food intake of the rats and thereby lowered the weight gain.

Within the interval 16-78 rpm, Riley (1981) found a linear relation between speed of rotation and plasma corticosterone concentration, indicating that anxiety stress in mice increased with the speed. When considering the higher body mass of rats, it seems reasonable to assume that significant reductions in the examined parameters could be induced in rats at rotation speeds over 78 rpm. This, however, would require a different experimental set-up involving other sources of rotation than a grammophone turntable, for instance the more torturous device described by Ohara *et al.* (1982).

After twenty days at 78 rpm, lymphocyte numbers and lymphoid tissue masses still tended to be reduced in rotated rats; but none of the differences were significant, suggesting that the animals during the experiment gradually adapted to the stress, thus lowering the anxiety level. This adaptation would make the regimen unsuitable for long-term stress experiments.

In conclusion, the present study indicates that body rotation by means of a grammophone turntable is a much less efficient stressor in rats than in mice. After four days a slight generalized lymphopenia is seen, which tends to disappear with time. Therefore, the method cannot be recommended for long-term experiments in rats.

Summary

Rotation stress induced by a grammophone turntable has profound effects on the immune system of mice. In the present study, we tested the effect of the method on the weight of lymphoid organs and cellularity of peripheral blood and lymph in rats.

After four days ($4 \times 5 \times 10 \times 78$ revolutions) the spleen weight was significantly reduced in rotated rats as compared to controls. Small, but not significant, reductions were also seen in body weight gain, thymus weight, inguinal lymph node weight, thoracic duct lymphocyte number and peripheral blood leukocyte number.

After twenty days ($20 \times 5 \times 10 \times 78$ rpm) the differences between rotated and control animals were smaller and not significant.

We conclude that the stress regimen induced a slight temporary reduction in total body lymphocyte number, but that the rats adapted to the situation with time. Consequently, the method cannot be recommended for long-term experiments in rats.

Sammenfatning

Rotationsstress ved hjælp af drejeskiven fra en grammofon påvirker immunsystemet voldsomt hos mus. Formålet med denne undersøgelse var at afprøve metodens virkninger på vægten af lymfoide organer og celletallet i blod og lymfe hos rotter.

Efter 4 dage ($4 \times 5 \times 10 \times 78$ omdrejninger) var miltvægten signifikant lavere hos roterede rotter end hos kontroldyr. Der sås også mindre, men ikke signifikante reduktioner i vægttilvækst, thymusvægt, vægt af inguinale lymfeknuder, lymfocyttallet i ductus thoracicus, samt leukocyttallet i perifert blod.

Efter 20 dage ($20 \times 5 \times 10 \times 78$ omdrejninger) var forskellene mellem roterede og ikke roterede dyr mindre og ikke signifikante.

Vi konkluderer, at den anvendte stress-metode medførte en beskeden midlertidig lymfopeni, men at rotterne relativt hurtigt vænnede sig til situationen. Metoden kan derfor ikke anbefales til langtidsforsøg hos rotter.

K. Pelkonen / Yhteenveto

Levysoittimen levylautasella aiheutetulla pyörimisstressillä on voimakkaita vaikutuksia hiiren immuunijärjestelmään. Tässä tutkimuksessa selvitimme menetelmän vaikutusta rotan imukudosten painoon ja veren ja imunesteen soluihin.

Neljän päivän jälkeen (4 × 5 × 10 × 78 kierrosta) pernan paino oli merkittävästi alentunut stressatuissa rotissa verrattuna kontrolleihin. Myös ruumiinpainon nousussa, kateenkorvan painossa, nivusten imusolmukkeiden painossa sekä rintatiehyen imusolujen ja ääreisverenkierron valkosolujen määrissä tapahtui vähäistä, mutta merkitsevää vähenemistä.
Kahdenkymmenen päivän jälkeen erot stressattujen ja kontrollieläinten välillä olivat pienempiä, eivätkä tilastollisesti merkitseviä.
Päättelemme, että stressitapahtuma aiheutti väliaikaisen imusolujen kokonaismäärän laskun, mutta rotat sopeutuivat ajan myötä tilanteeseen. Tämän vuoksi menetelmää ei voi suositella käytettäväksi rotalle pitkäaikaiskokeissa.

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