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Experimental set-up for detecting blood pressure, heart rate, and lymphocyte redistribution in the running rat

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Introduction

Physical activity as an immunomodulator has been investigated with increasing interest in recent years. In connection with physical activity various changes in the human immune system have been observed (*Escola* 1978, *Espersen* 1990, 1991). Recent investigations have also demonstrated alterations in the immune function in experimental animals in connection with physical exercise (*Arno* 1990, *Hofmann-Goetz* 1988, *Pahlavani* 1988, *Canon* 1984, *Ferry* 1990). One observation in humans has been a decrease in mononuclear white blood cells after exercise (*Espersen* 1990, *Ricken* 1990). In order to elucidate the fate of the lymphocytes, we designed an animal experimental set-up to investigate the redistribution of the mononuclear white blood cells in connection with physical activity. In principle, the basic set-up can be used in connection with the investigation of other cell types and blood parameters, and also function as a valid parameter in monitoring heart-rate and blood pressure in the rat.

Materials and methods

Animals

Male wistar rats bred at the Institute of Pathology, Aalborg Hospital for 22 years were used in the investigation (Mean age (min/max). Runners (n = 10): 133 (100/165) days. Controls (n = 10): 133 (100/165)). In this experimental set-up the animals were housed in the same room as the mechanical devices to make them accustomed to the environment.

Catheterization

The method was first described by *Popovic & Popovic* 1960 and modified as described by *Sonne* 1989.

The animal was anesthesized using intraperitoneal Mebumal (pentobarbital) and stesolid (Diazepam) (total: 25 mg and 0.1 mg respectively, approximately 8 mg and 0.3 mg per 100 g body weight). A catheter (Astra Meditec) with an outer diameter of 1 mm, an inner diameter of 0.5 mm and a total length of 22 cm was used in every trial. The catheter was rinsed and filled with heparin 100 IE per ml. We used a cannula (Terumo 26G × 1/2, 0.45 mm × 12 mm), which fitted the diameter of the catheter. The right carotid artery was dissected free and the catheter introduced into the artery. The tip of the catheter was placed approximately 2.2 cm distal from the insertion, approximately reaching the aortic arch. The catheter was secured with two ligatures, and after ensuring there was a flow of blood in the catheter, it was rinsed and filled to the tip with a mixture of heparin (500 IE) and Anhypen (Ampicillin) 250 mg (0.2 ml injected). The catheter was then closed using heat and compression. The closed catheter was tunnellised subcutaneously to a position in the neck region where another incision was made. The tip of the catheter protruded from the incision in the neck region and the rest was placed subcutaneously in a small pocket. The incision was covered by a small plaster to prevent the rat from damaging the catheter and itself.

In our experimental set-up the animals rested for three days before start of exercise.

Lymphocyte isolation and Indium^{111} labelling

One ml blood was drawn into heparin medium (RPMI 1640 + 30 IE heparin per ml). No antibodies were added. The sample was separated on Ficoll Hypaque (1.077 g per ml) for 20 min., (500 × G). Lymphocytes were harvested and washed in RPMI 1640 twice and the isolated lymphocytes were resuspended into 100 microl. of RPMI 1640. Labelling of the lymphocytes was performed using Indium^{111} (^{111}In), 0.8 microcurie (μCi) per 10^6 cells with an expected labelling efficiency of about 50 % resulting in about 0.4 μCi per 10^6 cells. Concentrations ranging between 0.2–0.4 μCi/10^6 cells are recommended. 2 1/2 hour after injection of lymphocytes labelled with these concentrations, the distribution of radioactivity has been shown to be close to that expected (*Sparshott* 1981). Furthermore, there was probably no reduced survival of the labelled cells within 2–3 hours (the critical level for 24 hours survival is 20 μCi/10^6 cells) (*Sparshott* 1981, *Garcia* 1988). Tropolone, which is a lipophilic compound, was added to ^{111}In thus forming a compound that readily enters the cell. Once inside the cell the compound becomes lipophobic and unable to pass through the undamaged cell menbrane. ^{111}In in 50 μl tropolone were mixed with 0.1 ml lymphocytes for 5 minutes during which the sample was shaken. Five ml. medium (RPMI 1640) was added followed by centrifugation for 10 min. (550 × G). Cellfree supernatant was then removed, and the remaining ^{111}In labelled cells were mixed with saline in a volume of exactly 500 μl which then was sucked into a syringe. A dosimeter measurement was performed on the supernatant and the syringe with the labelled lymphocytes. From the syringe 5 μl was taken to be measured in a gamma-counter. The rest of the material (495 μl) was injected into the rat (carotis catheter), and the catheter was finally rinsed and filled using a solution of antibiotics and heparin (0.2 ml, 500IE heparin and Anhypen 250 mg).

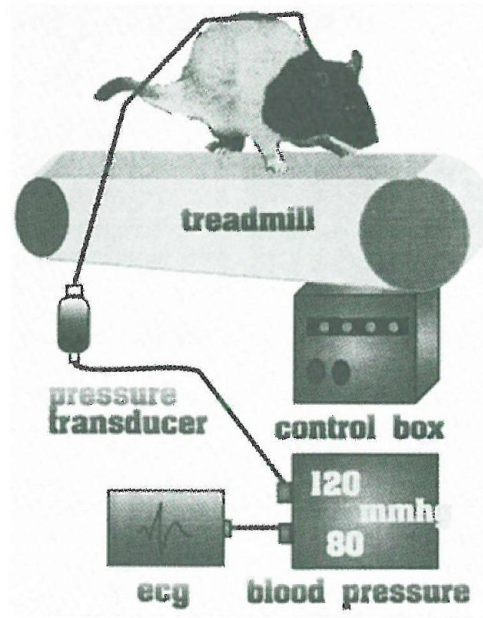

Figure 1. Schematical presentation of the experimental setup used in the investigation of lymphocyte redistribution in relation to physical exercise (see text for further details).

Monitoring of heart rate and blood pressure

The catheter (Astra, Meditec) (1 mm/0.5 mm) was connected to a Bell and Howell pressure transducer (type 4-327-C, range 0–400 mm Hg). The pressure transducer was connected to a neonatal monitor (Corometrics 512. Medical Systems inc. Wallingford, USA) for monitoring blood pressure, which was connected to a beat-to-beat heart rate monitoring device (Hellige, Germany). (See Fig. 1). The catheter connecting the catheter implanted in the carotid artery and the pressure transducer was not more than 20 cm long, but even this length might produce a too low systolic and too high diastolic blood pressure. Therefore, we recommend that the mean blood pressure be calculated.

Treadmill

A treadmill with possibilities of measuring speed (metres per second) and angle on the

running track was used (OM2, OM teknik, Copenhagen, Motor from Bauer, Germany). The treadmill had 12 running tracks (*Bech M.* 1989).

Experimental set-up

Having described the technical procedures in this set-up for determining lymphocyte redistribution and monitoring heart rate and blood pressure in the running rat, the total experimental set-up will be described.

All animals included in the study were allowed to run daily for several weeks before the start of the investigation to make them get used to the treadmill and enhance their general condition, since we assume that a certain amount of physical exercise is essential to demonstrate changes parallel to those found in experienced human runners in connection with competition or hard training. Three days before the day of investigation, one pair of animals (runner and control) had a catheter operated into the carotid artery as described. A 3 days resting period was chosen to allow the animals to recover from the anaesthesia and surgery. Cytokine levels, reflecting immune system activation, has been shown to increase, but return to normal within 1–2 days after operation (*Wells R. M.* 1992). Furthermore, the animals after 3 days all had a normal behaviour, judged by experienced personel. After 3 days, 1 ml of blood was drawn from each animal. The lymphocytes were harvested and labelled with ^{111}In as described. The labelled cell suspension was injected into the carotid catheter, which was rinsed and filled with heparin (100 i.e. per ml) afterwards. Before the "runner" rat started exercise and one hour after injection of the ^{111}In labelled cell suspension, resting heart rate and blood pressure were measured in the "runner" as well as in the control. The rat ran to exhaustion, defined as the time were the rats could not be induced to run anymore despite stimulation. Blood pressure and heart rate were measured in the exercising rat at the end of the run. At the same time the total running time and maximum speed were noted. We choose the speed 5 m $\cdot$ min^{-1} from start, gradually increasing the velocity every 3 minutes with 5 m $\cdot$ min^{-1}. After the run, the animals were killed, and dissection was performed. Tissue samples were collected, weighed and put into tubes for gamma counting. The gamma count values, corrected for tissue weight, were used to demonstrate any accumulation of difference between runner and control. When possible the whole organ was weighed (heart, liver, spleen, etc.). The weight of blood, bone marrow and lymph node was calculated using assumptions described by *Kaster* 1988 and *Garcia* 1988. The remaining tissue of the samples were fixed in a 4 % buffered formalin solution, and "running rats" and controls were simultaneously dissected. Specimens were prepared according to routine histological procedures and stained by hematoxylin and eosin. The histological examinations of the forma-

Table 1. Basic data from the experimental setup detecting changes in lymphocyte distribution, blood pressure and heart rate in the running rat (n = 10). Mean ± Standard Deviation (SD).

	"Runner"	SD	Control	SD
Weight (g)	297	67.2	310	48.4
Cells injected (10^6)	1.93	0.73	1.51	0.55
μCi/10^6 Cells	0.35	0.14	0.38	0.16
Mean Blood Pressure before (mm Hg)	114	1.94	111	3.66
Mean Blood Pressure after (mm Hg)	117	1.63	(no run)	(no run)
Heart rate before (beat/min)	393	27.2	405	31.2
Heart rate after (beat/min)	494	36.2	(no run)	(no run)
Running time (min)	18.9	6.68	(no run)	(no run)

lin fixated samples, which were blinded to the examiner, were made to investigate any visual accumulation of mononuclear white blood cells.
To demonstrate the accuracy of the setup, some basic data is shown in table 1.

Discussion
The catherisation technique and the routine of radioactive labelling of white blood cells, as well as the other different techniques used in this setup, have previously been described, but the experimental design presented here have to our knowledge never been reported before.
We find the described experimental set-up extremely useful and accurate in measuring heart rate and blood pressure in the exercising rat. Furthermore, it offers direct access to the blood stream, making it possible to examine different cells and biochemical parameters in the peripheral blood in close time relation to the exercise.

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Summary
An experimental set-up for detecting lymphocyte redistribution, blood pressure and heart rate in the running rat is presented.
The design is based on caterisation of the carotid artery which previously has been described. The design offers direct access to the blood stream making it possible to examine different cells and biochemical parameters.
Furthermore, continously monitorering of blood pressure and heart rate both at rest and during exercise is described.

Sammendrag
Der præsenteres en eksperimentel opsætning, der giver mulighed for undersøgelse af redistribution af mononukleære hvide blodlegemer og endvidere kontinuerlig monitorering af blodtryk og puls, under fysisk aktivitet i rotten.
Designet er bl. a. baseret på en tidligere beskrevet teknik til katerisation af arteria carotis. Der åbnes herved mulighed for direkte adgang til blodbanen og undersøgelse af forskellige cellulære og biokemiske forhold. Yderligere beskrives samtidig kontinuerlig monitorering af blodtryk og puls, såvel i hvile som under fysisk aktivitet.

Yhteenveto / K. Pelkonen
Raportissa esitellään menetelmä havainnoida rotissa lymfosyyttien uudelleenjakautumista, verenpainetta ja sydämen lyöntitiheyttä fyysisen rasituksen aikana. Tässä menetelmässä katetrisoidaan kaulavaltimo aiemmin kuvatulla tavalla ja päästään havainnoimaan ohivirtaavasta verestä eri solutyyppejä ja biokemiallisia muuttujia. Kuvatulla menetelmällä voidaan myös monitoroida sekä levossa että harjoituksen aikana.verenpainetta ja sydämen lyöntitiheyttä.

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