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Hematocrit changes and plasma loss during early endotoxin shock in rats

A.A. van Lambalgen, G.C. van den Bos and L.G. Thijs, Laboratory for Physiology, Free University, van der Boechorststraat 7, NL-1081 BT Amsterdam

We have studied the effect of plasma loss as a possible aggravating factor in the course of endotoxin shock in anesthetized rats. Hematocrit is continuously measured (conductivity cell) in an extracorporal circuit, simultaneously with activity of ^{51}Cr labeled red cells and ^{125}I-HSA in blood (multichannel analyzer). Shock was induced by *E. coli* endotoxin (10 $mg \cdot kg^{-1} \cdot h^{-1}$) infusion for 60 min starting at t = 0; at t = 105 min the experiments ended. Known amounts of ^{125}I-HSA and ^{51}Cr red cells were injected at t = −40 min. Control rats (n = 5) did not show gross changes in the measured variables. In endotoxin rats (n = 5) hematocrit increased from 42.1 at t = 20 to 45.5 at t = 45, but subsequently decreased to 42.0 at t = 105. Changes in ^{51}Cr activity in whole blood parallelled hematocrit changes. ^{51}Cr activity of red cells did not change during the experiment. Before endotoxin ^{125}I-HSA plasma activity decreased as in the control group between t = 20 and 45 it hardly changed, after t = 45 it further decreased. Trends were significant for the group. The initial transient rise in hematocrit was thus not due to increase of red cells but to plasma loss causing decreased blood volume. The subsequent decrease in hematocrit could be due to increased return of extravascular fluid to the circulation or to sequestration of red cells. Results indicate however that plasma loss did not cause a serious fall in circulating blood volume.

The influence of changes in cardiac output on arterial and mixed venous pH and pCO_2

Y.L. Hoogeveen, J.P. Zock, P. Rispens and W.G. Zijlstra, Department of Physiology, Bloemsingel 10, NL-9712 KZ Groningen

The net amount of CO_2 transported from the tissues to the lungs depends on blood flow, and on the arterial and mixed venous concentration difference. When the metabolic rate is constant, the CO_2 concentration difference is inversely proportional to cardiac output. Changes in cardiac output are therefore reflected in variations of the acid-base status of both mixed venous and arterial blood, even when arterial pCO_2 is kept constant (Roos, A., and Thomas, L.J. Jr, Anesthesiology *28* (1967) 1048). Using anesthetized, artificially ventilated dogs, we studied the influence of decreasing cardiac output while maintaining a near constant arterial pCO_2. The latter was controlled by means of ventilatory adjustments. Cardiac output was manipulated by applying positive end-expiratory pressure, and by β-adrenergic blockade to suppress the compensatory heart rate response. The systemic vascular response was attenuated by α-adrenergic blockade. Under these conditions a fall in cardiac output led to a shift in the plasma pH of arterial blood in the direction of a metabolic acidosis. The changes occurring in the mixed venous blood were less uniform; however, in general they represented a shift along the in vivo CO_2 bufferline as if a respiratory acidosis was developing. These results agree with the model calculations discussed by Roos and Thomas which show cardiac output as being one of the factors influencing in vivo CO_2 equilibria.

Opposing effects of central and peripheral chemoreceptors in the ventilatory response to metabolic acidosis

J.J. Schuitmaker, A. Berkenbosch, J. De Goede and C.N. Olievier, Department of Physiology, Leiden University, P.O. Box 9604, NL-2300 RC Leiden

We studied the normoxic ventilatory response to acute acid-base disturbances using the ABP technique in anesthetized cats. The ventilation was described by

$$\dot{V}_E = \alpha[H^+]_a^p + \beta[H^+]_a^c + \gamma Pa^c_{CO_2} - K \quad (1),$$

in which $[H^+]_a^p$ is the arterial H^+ concentration in the systemic circulation (peripheral), $[H^+]_a^c$ the arterial H^+ concentration in the blood perfusing the brain stem (central), $Pa^c_{CO_2}$ the central arterial CO_2 tension, α the peripheral H^+ sensitivity, β the central isocapnic H^+ sensitivity, γ the isohydric central CO_2 sensitivity and K a constant. The ratio of α to β was found to be about 2. Taking for the metabolic hyperbola:

$$\dot{V}_E = (P_B \cdot \dot{V}_{CO_2}) \cdot (Pa_{CO_2} - P_{ICO_2})^{-1} + \dot{V}_D \quad (2),$$

in which P_B is the barometric pressure, V_{CO_2} the metabolic CO_2 production, V_D the dead space ventilation, Pa_{CO_2} the arterial CO_2 pressure and P_{ICO_2} the inspiratory CO_2 pressure and linearizing eqs. (1) and (2) around the working point, $På_{CO_2}$, we find for small changes in the arterial H^+:

$$\Delta\dot{V}_E = (\alpha+\beta)[1-\gamma/\{\gamma+P_B \cdot \dot{V}_{CO_2}/(På_{CO_2}-P_{ICO_2})^2\}]\Delta[H^+]_a \quad (3).$$

It follows from our data that the increase in $\dot{V}$ following an acute metabolic acidosis, taking $P_{ICO_2} = 0$ consists of a positive contribution of the peripheral chemoreceptors while the central part is negative. For an acute metabolic alkalosis the reverse is true. After denervation ($\alpha = 0$), there is still a change in ventilation during acid-base disturbances which is about $1/3$ of the response in intact cats.

Influence of habituation to experimental conditions in acid-base experiments in conscious dogs

A.J.M. Langbroek, P. Rispens and W.G. Zijlstra, Department of Physiology, Bloemsingel 10, NL-9712 KZ Groningen

The use of conscious dogs in the study of ventilatory responses to changes in acid-base balance has advantages in comparison with anesthetized dogs in which the responses might be attenuated. However, conscious animals are influenced by experimental conditions such as renewed contact with its keeper causing excitement and hyperventilation. Because little information was available concerning the magnitude of the effect of hyperventilation on the acid-base balance, we measured the changes in arterial pH, pCO_2, pO_2, sO_2 and cHb in normal and acidotic conscious dogs with a permanent catheter in the aorta. Immediately after the dog entered the experimental room, it was placed in a basked where it remained quietly for 90 min. The first sample was taken within 2 min. Thereafter samples were drawn every 15 min. In normal and acidotic dogs pO_2 decreased significantly by 13.5 and 8.5 torr and sO_2 decreased significantly by 1.5 and 1.4%, respectively. Whereas pCO_2 in normal dogs remained nearly constant, in acidotic animals pCO_2 increased significantly by 2.6 torr. The largest changes in blood gas tensions and cHb occurred within 30 and 60 min, respectively. The total fall in cHb was circa 25 g/l, probably caused by relaxation of the spleen. The differences in blood gas tensions may be explained as the result of small changes in $\dot{V}/\dot{Q}$ inhomogeneity. The above described experiments have clearly shown that a period of habituation of

at least 60 min is required to obtain reliable data concerning the ventilatory response to acid-base disturbances occurring in conscious dogs.

Subendocardial underperfusion in the isolated, hypertrophic rat heart after 45 minutes normothermic, global ischemia

L.H.E.H. Snoeckx, G.J. van der Vusse, W.A. Coumans and R.S. Reneman, Department of Physiology, University of Limburg, P.O. Box 616, NL-6200 MD Maastricht

Isolated, working hearts of 16-month-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats were subjected to 45 min global ischemia, followed by 45 min reperfusion. The SHR- and WKY-hearts were divided into subgroups perfused at a diastolic afterload of 60 mmHg (SHR-60; WKY-60) and 100 mmHg (SHR-100; WKY-100).
During reperfusion recovery of cardiac output was completely absent in SHR-60 hearts (n = 7) and SHR-100 hearts (n = 6), while a 40% and 10% recovery was observed in the WKY-60 (n = 8) and WKY-100 (n = 6), respectively. Myocardial perfusion, as determined by radioactive microspheres before ischemia, after 15 min reperfusion and at the end of the reperfusion period indicated no reflow of the sub-endocardial layers in the SHR-60 group, while a marked underperfusion of the subendocardium, i.e. 61% of the subepicardial flow, was present in the SHR-100 group. Endocardial-epicardial flow ratios in both WKY groups were normal and not different from the pre-ischemic values. No-reflow in the SHR-60 group was also indicated by the high amounts of tissue lactate at the end of the reperfusion period (195 versus 40 $\mu mol \cdot g^{-1}$ in the WKY-60 group), and the higher amounts of breakdown products of energy-rich phosphates, i.e. 4.7 versus 1.8 $\mu mol \cdot g^{-1}$ in the WKY-60 group. These results show that at a comparable perfusion pressure, the hypertrophic rat heart has a diminished tolerance for myocardial ischemia, associated with a decreased or absent perfusion of the subendocardium, followed by tissue accumulation of breakdown products of myocardial metabolism.

Intravital microscopic observations of rat pial microcirculation

W.E. van Bree, G.J. Tangelder, D.W. Slaaf and R.S. Reneman, Laboratory for Microcirculation, Departments of Physiology and Biophysics, University of Limburg, P.O. Box 616, NL-6200 MD Maastricht

An open skull model has been developed that allows microscopic investigation of microvessels at the surface of the brain in rats in vivo.
Five Wistar Kyoto rats were anesthetized with α-chloralose+urethane (1.0+13.3%; 6 ml/kg) through a preimplanted intravenous catheter. The use of ether was avoided because this facilitates the development of brain swelling. The animals were allowed to breath spontaneously through a tracheal tube. The head was mounted in a stereotactic apparatus. A rectangular cranial window (5 × 7 mm) was made in the parietal region, using a low-speed drill and cooling by saline drip. Bone rongeurs were not employed because this often causes excessive brain swelling. Bone bleedings were controlled by bone wax. The dura was raised with the bended tip of a 25G needle and then cut with sharp ophthalmic scissors. Dura bleedings were stopped by a fine ophthalmic cauter and spongostan. The brain surface was covered with a pool of artificial cerebrospinal fluid (depth > 1 cm). Microvessels were observed by intravital video microscopy (Leitz Ultropak system). After an observational period of 1 h brain swelling was still limited.
Within the cranial window one or two end-branches of the medial cerebral artery could be observed, that bifurcated several times and finally either penetrated the cortex or branched into superficial cortical capillaries. Arteriolar diameters ranged from 10–56 μm. Upon carbondioxyde inhalation all arterioles dilated (42–145%). The number of venules (diameter range 10–292 μm) was larger and their branching pattern more variable than that of arterioles.

Thromboembolic reactions in microvessels in vivo following wall puncture

M.G.A. oude Egbrink, G.J. Tangelder, D.W. Slaaf and R.S. Reneman, Laboratory for Microcirculation, Departments of Physiology and Biophysics, University of Limburg, P.O. Box 616, NL-6200 MD Maastricht

In the present study the reaction of blood to mechanical injury of the vessel wall was investigated in arterioles and venules (diameter range 20–40 μm) of the rabbit mesentery, using intravital video microscopy. A micro-trauma was inflicted by puncturing the vessel walls with glass micropipets (tip diameters 6–8 μm). Following wall puncture the growth of thrombi, which consisted mainly of platelets, started immediately. Formation of emboli continued significantly longer in venules (median value (M): > 600 s, being the maximal observation period) than in arterioles (M: 110 s). The median number of emboli produced during these periods was 35 in venules and 6 in arterioles. Diameters and red blood cell velocities in arterioles (M: 28 μm and 2.2 mm/s) did not differ significantly from the values found in venules (M: 30 μm and 1.8 mm/s), indicating that the observed difference in thromboembolic reactions between arterioles and venules cannot be explained by hemodynamic factors. To investigate whether arachidonic acid derivates are involved, the cyclooxygenase inhibitor indomethacin was added to the superfusate (10 μg/ml). In both venules and arterioles the duration of embolization (M: 113 and 56 s, respectively) and the number of emboli produced (M: 8 and 2, respectively) were diminished. This suggests that arachidonic acid derivates, although involved in platelet-vessel wall interactions after injury, are not completely responsible for the differences in thromboembolic reactions between arterioles and venules as observed in vivo.

Capillary recruitment does not occur in young rabbit tenuissimus muscle

H.H.E. oude Vrielink, D.W. Slaaf, G.J. Tangelder and R.S. Reneman, Laboratory for Microcirculation, Departments of Physiology and Biophysics, University of Limburg, P.O. Box 616, NL-6200 MD Maastricht

Following vasodilation, skeletal muscle blood flow may increase 10–15 times as compared to the resting situation. Whether this enhanced blood flow can be attributed to capillary recruitment, i.e. perfusion of an increased number of capillaries, has been investigated. Sixteen young mixed-breed rabbits (5–6 weeks; 950–1150 g) were anesthetized (20% urethane; 10 ml/kg i.v.). The tenuissimus muscle was exposed (Reneman et al., MVR *20* (1980) 307) and the vascular bed was investigated by intravital microscopy in a low oxygen environment. Within the muscle, several transverse arterioles (TA) are fed by a central artery. The first order side branches (FOS) of these TA show extensive vasomotion and in this way control the (intermittent) perfusion of the downstream capillaries. We studied whether recruitment occurred by perfusion of previously non-perfused FOS or by perfusion of individual capillaries not perfused in the resting situation. FOS were visualized by fluorescence microscopy after injection of 5% FITC-dextran. Vasodilation (9×10^{-5} M adenosine applied topically) did not increase the number of FOS perfused. In another set of experiments, using bright field mi-

croscopy, the number of capillaries perfused by the same parent FOS did not increase after vasodilation (reactive hyperemia). During vasodilation capillary flow became continuous in all experiments. These findings indicate that an enhanced blood flow in young rabbit tenuissimus muscle is *not* achieved by capillary recruitment, but by a transition from intermittent to continuous capillary perfusion. Supported by FUNGO/ZWO.

Estimation of the time constant of vascular volume change in the coronary circulation

I. Vergroesen, M.I.M. Noble and J.A.E. Spaan, Departments of Physiology and Physiological Physics, Leiden

The vascular volume change in the first moments of cardiac arrest was estimated by integrating the difference between arterial inflow and corrected great cardiac venous outflow. In nine anesthetized goats the left main coronary artery was perfused under pressure control. The venous flow signal was amplified so that the calculated intramyocardial blood volume was constant in the beating heart. With normal vasomotor tone 1.60 ± 0.09 s (mean ± SE) were required to achieve 67% of the volume change (3.04 ± 0.18 ml/100 g LV) and in the fully dilated coronary bed (adenosine infusion) these values were 0.96 ± 0.06 s and 4.13 ± 0.33 ml/100 g LV respectively. These time constants are long compared to the duration of diastole in the beating heart. A model of the coronary circulation was used to locate the positions of the volume change (compliance) in relation to the resistance distribution of the coronary bed. From the combination of the model analysis and the measurement we conclude that 1) the measured volume change is not in the epicardial veins and 2) the microvasculature is capable of a substantial but slow volume expansion when myocardial compression by ventricular systole is suspended.

Effects of myocardial blood flow on electrical conductivity of heart muscle

P. Steendijk, A.D. van Dijk, J. Karsdon, M. Marcus and J. Baan, Clinical Physiology Laboratory Department Pediatrics, Leiden University Hospital, Leiden

Electrical conductivity of the myocardium is known to be anisotropic and dependent on muscle fiber direction. To be expected, but not yet investigated, is the influence of blood in the capillary vessels on the electrical properties of the myocardial wall. We developed an electrode system consisting of two perpendicular arrays of four electrodes with an inter-electrode distance of 1 mm. The electrode system is incorporated into a small flexible silicone suction cup and the sensor can be affixed to the epicardium using a slight vacuum, resulting in very little tissue damage. Using a four-electrode method (applied current: 10 μA, 15 kHz), conductivity is calculated from voltages measured in two perpendicular directions. In this configuration conductivity measurements are limited to an epicardial layer of about 2–3 mm. In 3 anesthetized dogs we positioned the sensor in the perfusion region of the left anterior descending coronary artery (LAD) and studied wall conductivity while monitoring coronary flow with a flow probe on the LAD. Changes in coronary flow were induced by occluding the aorta or the LAD. Under normal flow conditions, conductivity ranged from 0.30 to 0.42 S/m in the fiber direction ($\sigma_\parallel$) and from 0.22 to 0.28 S/m perpendicular to fiber direction ($\sigma_\perp$). When increasing myocardial flow by aortic occlusion, $\sigma_\perp$ increased slightly and $\sigma_\parallel$ showed a strong decrease (typically −15%). Decreasing flow by LAD occlusion yielded a considerable decrease in $\sigma_\perp$ (−12%) and dented to increase $\sigma_\parallel$. After removal of either occlusion $\sigma_\parallel$ and $\sigma_\perp$ returned to their original values. As these effects appear to reflect regional myocardial blood flow, this technique is concluded to be promising.

Segmental left ventricular (LV) volumes by conductance catheter compared to volumes by cine computed tomography (CT)

A.D. van Dijk, E.T. van der Velde and J. Baan, Clinical Physiology Laboratory, Department of Pediatrics, Leiden University Hospital, and L. Diethelm, S.A. Glantz, M. Lipton and Ch.B. Higgins, Departments of Radiology and CVRI, University of California, (UCSF) San Francisco

The conductance catheter (CC) has previously been shown to give reliable measurements of total LV volume (V) both in situ and in the isolated heart (Baan, C.S., Circ. *70* (1984) 5; Circ. *72* (1985) 2. We compared segmental V's from the CC and from CT in the anesthetized closed chest dog preparation. The CT technique at UCSF enabled us to obtain segmental cross sections of the LV at 8 levels perpendicular to the long axis at ten 50 ms intervals (total 500 ms) using an i.v. injection of contrast medium. Simultaneously, 5 segmental V's were obtained by the CC method at comparable LV levels. Following computer reconstruction of the CT images, contours of each frame were detected using a semi-automatic edge detection technique. Time-dependent V's of the 5 LV segments throughout systole and early diastole from the two methods were submitted to linear regression (LR) analysis.

Results analyzed from 4 dogs thus far show that the two methods are highly correlated (r = 0.88 to 0.99, median: 0.95) in the 4 levels of the LV from apex to above equator. Correlation at the outflow tract level was generally poor, partly caused by the inability to detect the position of the mitral valve in the CT method and possibly by atrial components to the CC signal at the level. At the same 4 levels, average offsets in absolute V were low (1–7 ml) while average slopes of the LR were close to expected values (0.74–1.07). Conclusion: both methods have great potential in measuring segmental V's of the LV and their contributions to stroke volume and ejection fraction.

Lattice structure and the calcium sensitivity of skinned papillary and skeletal muscle of the rabbit

E.L. de Beer, B. Wilhelm, C. Caljouw, R.L.F. Gründeman, F.A.M. van Kaam and P. Schiereck, Department of Medical Physiology and *Department of Pharmacy, State University of Utrecht, Vondellaan 24, NL-3521 GG Utrecht*

Calcium activated isometric tension development was studied in isolated papillary and single skeletal muscle fibers (M. Gracilis) of the rabbit. The fibers were skinned by means of the freeze-drying method as described by Stienen et al. (Pflügers Arch. *397* (1973) 272). The experiments were conducted at room temperature (20 °C). The composition of the solutions was calculated according to Fabiato and Fabiato (J. Physiol., Paris *75* (1979) 463).

Sarcomere length of the fibers was measured during the experiment using laser diffraction techniques. It varies during the contraction less than 0.02 μm. The calcium sensitivity was studied at sarcomere length between 1.2 and 2.3 μm (papillary) or 3.6 μm (skeletal). Activation curves were plotted relating force to pCa (range 7.0–4.5) at different sarcomere lengths. In addition the muscle was compressed by adding 5% of dextran to the bathing solution. Force was developed over the whole range of observed sarcomere lengths. At a sarcomere length of 1.2 μm developed force equals 25%–35% of the force at 2.25 μm.

Both stretching either type of muscle and osmotic compression of the fiber result in shift of the normalized pCa-Force curves.

Stretching results in a shift to lower pCa-values, while osmotic compression results in a shift to higher pCa values. The present results indicate that the exact position of the normalized pCa-Force curve is not only dependent upon actin-myosin overlap but also upon interfilament spacing.

Toward a sarcomere basis for heart muscle contraction

F. A. M. van Kaam, E. L. de Beer and P. Schiereck, Department of Medical Physiology, Vondellaan 24, NL-3521 GG Utrecht

Recent fundamental studies on heart muscle contraction show several of the features of the contractile mechanism which are more generally known for striated muscle preparations (van Kaam et al., Archs int. Physiol. Biochem. *91* (1983) 451; van Kaam et al., Circ. Res., (1986) in press). During steady volume release an intact left ventricle shows a shoulder shaped pressure transient dependent upon the velocity of release comparable with the force transients seen during release of tetanized skeletal muscle fibers (Ford et al., J. Physiol. *269* (1977) 441). During steady volume increase, pressure enhancement shows a decline comparable with the decline in force enhancement in tetanized skeletal muscle fibers (Edman et al., J. Physiol. *281* (1978) 139) and in contracting trabecula during controlled steady sarcomere stretch (Krueger and Tsujioka, in: Some Mathematical Questions in Biology, Ed. R. Miura, (1985) in press). These similarities are all the more remarkable in view of the complex geometry, anisotropy and inhomogeneity of the ventricle. During contraction, activation is continuously changing as a function of time. This can be rather well incorporated in a modified multi-state cross-bridge model (Julian et al., Biophys. J., *14* (1974) 546; Huxley and Simmons, Nature *233* (1971) 533; van Kaam et al., Proc. 8th int. Biophys. Cong. IUPAB, (1984) 205). Breakage of cross-bridges by stretching beyond a certain length has to be incorporated to explain the decrease in pressure during stretch (van Kaam et al., thesis, Utrecht 1985). From these present studies we have obtained strong evidence that the mechanical behavior of the isolated ventricle reveals more properties of the contractile mechanism than one might expect.

Tyrosine metabolism in protein deprivation

V. V. A. M. Schreurs, Z. Hofman, H. A. Boekholt and R. E. Koopmanschap, Department of Animal Physiology, Agricultural University, Wageningen

To maintain its nitrogen balance an organism requires a daily protein intake to replace the amino acid loss inherent to the turnover of proteins. The loss of amino acids is mainly due to their catabolism. The catabolism of tyrosine is initiated by a deamination followed by an irreversible decarboxylation. The remainder of the molecule is further metabolized to fumarate and acetoacetyl-CoA. Both metabolites follow different metabolic pathways dependent of the nutritional status. This study describes tyrosine metabolism in rats at three levels of protein intake. The protein content of the three diets was 20, 10 and 0% of total energy. After four weeks of adaptation to the diets the animals were injected with radiolabeled tyrosine. Two types of tyrosine were used; 1-14C and U-14C. The decarboxylation and further oxidation of tyrosine can be followed as the expiration rate of radioactive carbondioxide. All the injected radioactivity seemed to be cleared from the circulation in about 4 h. This means that it is either catabolized or incorporated in protein. On a normal diet the expiration of radioactive carbondioxide was significantly higher for 1-14C compared to U-14C tyrosine. This indicates that part of the molecule was retained by the body after decarboxylation, most probably for the formation of body fat from the excess of protein. When the protein content of the diet dimininishes the level of amino acid oxidation decreases while the values for the two types of tyrosine converge. The results indicate that protein deprivation reduces irreversible decarboxylation of tyrosine. However, when decarboxylation has taken place the remainder of the molecule is immediately further oxidized.

Oral contraception in athletes. Effect on protein C, protein S, C4b-binding protein and t-PA

I. A. Huisveld, J. E. H. Hospers, W. B. M. Erich and B. N. Bouma, Departments of Physiology and Hematology, State University Utrecht, Utrecht

The effect of oral contraceptives (OCA) on the various proteins was studied in 20 highly trained competitive rowers. Ten females (users) were on (low-dose estrogen) OCA-medication, ten others served as controls. The two groups were highly comparable with respect to age (23.7 ± 1.8 and 23.4 ± 2.2), body composition (% bodyfat: 24.7 ± 4.3 and 23.3 ± 2.5) and physical conditioning (VO_{2max}: 3.84 ± 0.44 and 4.18 ± 0.32). Protein C (PC), protein S (PS) and C4b-binding protein (C4b-bp) antigen levels were determined in plasma by rocket immuno electrophoresis and expressed as % of a normal pool. Tissue-type plasminogen activator (t-PA) antigen was determined by ELISA and t-PA activity was measured using S2251 as a substrate. Blood samples were obtained during morning hours in the 1st half of the cycle. Stimulation of the fibrinolytic mechanism (i.e. increase in t-PA) was assessed after standardized exhaustive exercise on a bicycle ergometer. Comparison of pre-exercise data, revealed significantly lower levels in users of both PS (84.5 ± 19.0 against 111.7 ± 21.7 in non-users) and C4b-bp (81.5 ± 14.0 against 96.4 ± 13.8 in non-users). No significant influence of OCA on PC or t-PA-ag levels was observed. Post-exercise increments in t-PA-ag and t-PA-act were of a comparable magnitude in both groups. PC-, PS- nor C4b-bp-ag levels do seem to have a predictive value with regard to fibrinolytic potential, since only weak correlations were observed between the pre-exercise levels of these proteins and the post-exercise fibrinolytic (t-PA) response.

Species differences in the response of serum cholesterol to the type of fat in the diet

A. C. Beynen, Department of Laboratory Animal Science, State University, P.O. Box 80.166, NL-3508 TD Utrecht

Dietary polyunsaturated fatty acids cause a decrease of serum cholesterol concentration in man, when compared to saturated fatty acids. We have studied this effect in laboratory animals. In rabbits fed semipurified diets we observed that the isocaloric replacement of saturated fatty acids (coconut fat; 9%, w/w) by polyunsaturated fatty acids (corn oil) lowers serum cholesterol levels, irrespective of whether the diets contained cholesterol (0.1%, w/w) or were cholesterol-free. In rats fed cholesterol-free diets, polyunsaturated fatty acids in the form of corn oil (20%, w/w) were found to increase serum cholesterol concentrations, when compared to saturated fatty acids given is coconut fat. The opposite effect however, was seen when the diets contained high amounts of cholesterol (1%, w/w). In mice dietary corn oil (20%, w/w) lowered serum cholesterol compared to coconut fat, but the difference became much smaller when high-cholesterol (1%, w/w) instead of cholesterol-free diets were used. Thus animal species may differ in their response of serum cholesterol to dietary fatty acids. Since the hypocholesterolemic action of polyunsaturated fatty acids in man is only poorly understood, comparative studies may be of great importance in order to shed more light on the mechanisms involved.

Entrainment in sinusoid light cycles and the Aschoff Curve in the albino rat: a VRC-based model of entrainment

J. Ruis, Department of Physiology, Division of Medical Chronobiology, University of Leiden, Leiden

Male Wistar rats (n = 4) were exposed to sinusoid varying light intensity cycles (T = 24 h) at various amplitude and intensity levels while recording locomotor activity and food intake continuously and automatically for about 10 months. It appeared that rats entrained to very small amplitudes at low intensity levels but failed to entrain to small amplitudes at higher intensity levels.
In a second set of experiments male Wistar rats (n = 8) were placed in constant light conditions of several intensities while the period of the freerunning rhythm in locomotor activity and food intake was measured. An Aschoff Curve was obtained that showed a high dependence of period on light intensity within a certain range of environmental illumination but showed saturation at higher light intensities.
A mathematical model, based on velocity response curves, was developed and the algorithm programmed on a DEC PDP 1170 computer. Analysis of the data showed that the Aschoff Curve offered an explanation for the entrainment in sinusoid lightcycles. Furthermore it appeared that a parameter of the model deduced from the Aschoff Curve was consistent with theluminance coding of visual responsive cells in the suprachiasmatic nuclei, as has been described by other investigators. The model could also adequately simulate a number of other experiments published in the literature. In conclusion, it can be said that the parametric effects of light, mediated by the properties of visual responsive cells, play an important role in the entrainment of circadian rhythms to light cycles.

Luminance coding of the circadian system

J.H. Meijer[1] and G.A. Groos[2], [1]Biological Center, University of Groningen, Haren and [2]Department of Physiology, Leiden University, Leiden

In mammals the suprachiasmatic nuclei (SCN) of the hypothalamus function as a dominant circadian pacemaker. This pacemaker is entrained by the external light-dark cycle via the retina. In constant darkness phase advances and delays of the freerunning circadian rhythm are observed depending on the phase of the circadian cycle at which light is presented. In 18 male adult hamsters the magnitude of a phase advance shift was studied as a function of the light intensity. After a release in continuous darkness for a period of 7 days 15-min white light pulses were presented 6 h after activity onset (CT = 17.6 ± 0.6, N = 77). The relation between the light intensity (lux) and the magnitude of phase shift could be described with a sigmoid shaped curve. Below 0.1 lux no phase shifts were observed while the working range is about 3 log units. These results are consistent with the intensity response (I.R.) relation previously described by J. Takahashi et al. (Nature *308* (1984)) for monochromatic light. Extracellular single unit recordings in the hamster SCN revealed that a subpopulation of visually responsive SCN cells also code for luminance with a sigmoid shaped I.R. curve (J.H. Meijer et al., Brain Res.,. (1986) in press). However, the threshold of the visual cells (10 lux), is high compared to the threshold for phase shifts (0.1 lux). The relative high threshold of the visual cells may be attributable to the use of anesthetics in the electrophysiological experiments or to the fact that only the steady state discharge rate was plotted as a function of light intensity. The observation though that both I.R. curves exhibit similar shapes suggests that the intensity dependent phase shifts of the circadian rhythm are mediated by the visual cells in the SCN.

Electrophysiological changes in catfish *(Ictalurus nebulosus)* electroreceptor functioning after denervation

R.C. Peters, E.L. Groen, M.M. van der Sluis and K.A. Wilhelm, Laboratory of Comparative Physiology, University of Utrecht, Jan van Galenstraat 40, NL-3572 LA Utrecht

The electrophysiological activity of single electroreceptors of catfish can be recorded in vivo under Saffan® anesthesia by means of a non-invasive technique. We investigated the effects of cutting the afferent nerve (n. opht. superficialis VII) on the electrophysiological properties of the electroreceptors in the rostro-dorsal skin. 80 electroreceptors in 8 fish were examined. The experiments were performed at 15–17 °C. We found the amplitude of the action potentials to decrease and the impulse activity to disappear within 48 h after denervation. The spontaneous activity decreased from 40 to 25 Hz (after 24 h). The slope of the input-output characteristic (sensitivity) did not change but a threshold seemed to develop. The frequency response, measured from 3 to 40 Hz, did not show any significant changes. The effects found might be explained by the shrinking of the synaptic contracts as described by Szamier and Bennett (J. Cell Biol. *56* (1973) 466).

Ampullary electroreceptors in catfish *(Ictalurus nebulosus)*: an alternative for the LD 50?

R.C. Peters and E. Hensbergen, Laboratory of Comparative Physiology, University of Utrecht, Jan van Galenstraat 40, NL-3572 LA Utrecht

Catfish electroreceptors are extremely sensitive to electric currents (nA/cm^2) and to fluctuations of the ionic composition of water. The electrophysiological activity of single electroreceptors can easily be recorded in vivo by a non-invasive technique. We exposed small pieces of skin on which several electroreceptors to low concentrations of $CuSO_4$ and $CdCl_2$ under Saffan® anesthesia for 1 h. In this way we obtained data from 12 single electroreceptors (6 controls, 6 tests) in 6 specimens. The electrophysiological activity of each individual receptor was measured 48 times during 5 days. Application of 0.1 mM $CuSO_4$ abolished the normal functioning of the receptor within 1 h; recovery afterwards took at least 3 days. Application of 0.1 mM $CdCl_2$ abolished the receptor functioning within 30 min; partial recovery took more than 4 days. We conclude that the electroreceptor is a very promising system for electrophysiologically monitoring the effects of heavy metals on nervous tissue.

Development of the ERG in a number of normal and mutant mouse strains

J.H. Reuter, Department of Physiology I, Faculty of Medicine, Erasmus University, P.O. Box 1738, NL-3000 DR Rotterdam

The ERG was recorded from mice, both albino and pigmented, at the age of 14, 21, 28 days and 2 months. The strains tested were: Balb/c^{++}, C3H^{++}, C3Hrdrd, 020rdsrds and C3Hrdrd/rdsrds. ERG's were recorded between an electrode in the anterior eye chamber and one over the eye. The stimuli were produced by a Grass PS2 stimulator and were attenuated by neutral density filters. Recording was started at the lowest intensity, which was then increased in steps. The threshold of waves was taken as the intensity at which the wave was clearly discernable. Amplitude measurement was conventional. At the start of the 24 h dark adaptation, atropine eyedrops were instilled in the

eyes. Preliminary results will be presented on the changes of both the a- and the b-wave, and on those of the threshold with age. The preliminary results show that the amplitude increases between days 14 and 21 in the normal strains, but decreases slightly after that. Differences in absolute amplitude were found between albino and pigmented strains. In the rdrd mice the amplitude decreased with age, but in the rdsrds mice the amplitude increased, in the rdrd/rdsrds mice a small ERG was found only at day 21.

Effects of perinatal hypoxia on visual development in infants born after different gestational periods

F. Groenendaal, G. Mohn, W.P.F. Fetter, W. Baerts* and J. van Hof-van Duin, Department of Physiology I, Erasmus University Rotterdam, and *Department of Neonatology, University Hospital Rotterdam, Rotterdam*

Perinatal hypoxia is generaly recognized as a major cause of physical and mental handicap, and can lead to severe visual defects (Van Hof-van Duin, J., and Mohn, G. Behav. Brain Res. *14* (1984) 147). Since the acute effects of hypoxia on the brain vary with the gestational age of the infant at birth, the question arises whether this might also affect the functional consequences. This report describes the results of a retrospective study of visual development in 124 infants born after gestational periods of 26–44 weeks, who experienced perinatal hypoxic events as indicated by various clinical measures. Visual acuity, visual field size, optokinetic nystagmus, and the visual threat response, as well as ocular motility and strabismic deviations were assessed at 3, 6, 9, and/or 12 months after the expected date of term.
Defects of binocular visual functions were seen in 52% of all infants at one or more examinations, with the highest proportions among very premature (gest. age 26–30 weeks) and fullterm infants ($\geqslant 37$ weeks). Visual development was often delayed during the first months after term and tended to improve with age, although there was no statistically significant decrease of actual defects in either preterm or fullterm infants. The results fail to provide clear evidence for a differential effect of perinatal hypoxia on visual development during the first year in infants of different gestational periods.

Human smooth pursuit: Effects of stimulus extent and of spatial and temporal constraints of the pursuit trajectory

A.V. van den Berg and H. Collewijn, Physiology I, Erasmus University Rotterdam, NL-3000 DR Rotterdam

Previous investigations have shown that the gain of the smooth component of both optokinetic nystagmus (OKN) and pursuit are a decreasing function of the velocity of the visual stimulus. The fall-off was steeper for pursuit than for OKN. The use of stimuli of different extent in these experiments precludes the conclusion that the difference in fall-off reflects a difference in the control systems involved. It is well known that the gain of OKN increases when the stimulus extends over a larger part of the visual field. However, the influence on smooth pursuit of the extent of a textured stimulus is unknown. We compared the pursuit of a full-field striped pattern, a point target and their combination at target velocities between 9 and 90 deg/s. A point target moving in a fixed trajectory maximally constrains target selection as well as the pursuit trajectory, whereas a full-field multi-contoured pattern leaves the subject maximal freedom in these respects. To unconfound effects of pattern extent from those of spatial and temporal constraints, we presented point targets under conditions in which the subject was free to choose the location, extent and temporal structure of his pursuit trajectory ('free range'). This was accomplished by stabilizing the target on the fovea during saccades that carried the eye back to the midposition. Pursuit velocity gains were lowest for the point target moving in a fixed trajectory. Gain improved when the subject was free to pursue the same target moving at the same velocity in his own preferred range and rhythm. A further improvement was obtained by showing the stripe pattern in addition to, and moving in conjunction with the spot. A final increase in gain occurred when the spot was removed, and the subject was allowed to choose any feature of the uniformly moving pattern as the momentary target for pursuit. In conclusion, pursuit of a target moving at a constant velocity may be constrained by 1) the target trajectory, 2) a small number of moving contours, 3) the selection of a detail rather than the whole pattern for pursuit.

Vergence eye movements induced by large disparities in open-loop conditions

C.J. Erkelens, Physiology I, EUR, NL-3000 DR Rotterdam

Experiments under open-loop conditions have made important contributions to the understanding of the relation between binocular disparity and ocular vergence. Experimental difficulties have restricted such measurements mainly to small disparities. Open-loop experiments were carried out in which disparities up to 10 deg were imposed and ocular vergence angles up to 40 deg were reliably measured. Movements of both eyes were recorded with scleral coils. Half of the ocular vergence signal was fed to each of the two mirrors that controlled the horizontal movements of the half-images in order to create open-loop viewing conditions for vergence while keeping normal feedback for version. First, stepwise changes of disparity in the crossed direction with amplitudes between 0.5 and 10 deg were imposed. Ocular vergence responses consisted of converging movements with an initially constant velocity. This velocity increased with the size of disparity up to about 4 deg but decreased for larger disparities. For disparities up to 2 deg convergence was sustained; For disparities above 2 deg responses were different: (a) they were transient, i.e. after the initial movement the angle of convergence declined to its initial value; (b) the maximal amplitude of the convergence decreased for larger disparities; (c) complete absence of response occurred occasionally at disparities of 6 deg and more. Disparity pulses with durations ranging from 0.1 to 3.2 s and interpulse intervals from 0.25 to 5 s induced vergence movements following the time integral of disparity rather than momentary disparity. These results indicate that the part of the vergence system sensitive to disparity has mainly integrative properties for large as well as small disparities. In addition the possible significance of the verlocity of the disparity change was examined. Preliminary results suggest that conditions of decreasing crossed disparity may induce an arrest of converging eye movements before disparity reaches zero. The proposition is made that this behavior, that has been attributed to prediction of stimulus motion, is better explained by responsiveness of the vergence system to velocity of disparity change.

The effect of unilateral lesions of the sensory-motor cortex as a function of age

J.F. Hobbelen, M.W. van Hof and W.H. de Vos-Korthals, Department of Physiology I, Erasmus Universiteit Rotterdam, P.O. Box 1738, NL-3000 DR Rotterdam

In the rabbit a unilateral ablation of the occipital lobe severely impairs pattern discrimination with the eye contralateral to the lesion. The defect was found to be as severe in rabbits operated 1–3 days and 3 months after birth. In the present experiments it was studied whether this age independency also applies to the motor system. It was found that 4 months after the operation the hopping reaction in the leg contralateral to the lesion was still absent in animal operated in adulthood. In animals operated 1

day after birth a normal hopping reaction was found when tested 1.5 and 4 months after the operation (Hobbelen, J. F., and van Hof, M.W., Behav. Brain Res., (1986) in press). Preliminary results indicate that a normal hopping reaction develops when the lesion is made before the end of the fourth week after birth.

Reversal of the sleep wake cycle in the rat: Effects on sleep stage patterns, EEG and other physiological rhythms

M. Lancel, Department of Physiology and Physiological Physics, Division of Medical Chronobiology, University of Leiden, Leiden

Structural and intensity changes in sleep are very similar for man and rat. For instance, during normal sleep the amount of slow wave sleep (SWS) decreases, as does its spectral power density (SPD) in the lower frequency range, while the amount of REM-sleep increases. When sleep is shifted by 12 h, it is shortened at the expense of light sleep and REM-sleep. Because of the similarities in both dimensions, the rat appears a promising model for the study of the effects of sleep-wake reversal on sleep. The purpose of this study is to examine the effects of sleep-wake reversal on sleep parameters, with an emphasis on SPD-changes in the sleep stages and wake, in relation to changes in the rhythms of body temperature and eating.

Four rats were used as experimental animals in two experiments, under a 12-h light/12-h dark schedule. The duration of both experiments is 13 days, i.e.: 4 adaptation days, 1 control registration day, 5 experimental days and 3 recovery days. During the experimental days in experiment 1 (day-shift) the rats were sleep deprived (by placing them in a rotating cylinder) in the dark period and in experiment 2 (night-shift) the rats were sleep deprived in the light period. Cortical EEG, neck muscle EMG, temperature and eating behavior were continuously recorded. During the night-shift, preliminary inspection of the eating and temperature data suggests the presence of a masking effect, caused by activity in the light period. During the day-shift the 3-h pattern in temperature becomes more evident in the dark period, while the temperature in the light period flattens. The amounts and distribution of the vigilance stages of 2 rats in the day-shift remain broadly the same, however, the number of stage-switches decreases substantially. All stages show higher SPDs in the lower frequency range in the first hours of sleep. The SPD values for the recovery light period in wake and REM sleep were normal and for NREM-sleep lower.

The effect of neonatal noradrenaline depletion on the spontaneous bioelectrical activity of single cells in the cerebral cortex of the anesthetized rat

F. Dijcks, M. Mirmiran, E. Brenner, M. Corner and F. Habets, Netherlands Institute for Brain Research, Meibergdreef 33, NL-1105 AZ Amsterdam

Experimental studies on the role of noradrenaline in the development of the CNS of the rat have shown that intraventricular injection of 6-OHDA on post-natal day 12 reduces the adult cortical noradrenaline level to ± 30% of the control value. This treatment also reduces cerebral cortical growth and the rate of response in certain behavioral tasks. That most of the noradrenergic projections from the locus coeruleus to the occipital cortex had in fact been destroyed by the 6-OHDA treatment was shown by means of retrograde tracing using cortically injected WGA-HRP. In order to investigate the physiological significance of chronic cortical noradrenaline depletion during development, the spontaneous activity of single cortical neurons was studied by means of quantitative spike train analysis. Extracellular recordings lasting 15 min were obtained with HRP-filled micropipettes from 10 male Wistar rats weighing 500–650 g (10–12 months old) under urethane anesthesia. On line acquisition of 36 neurons was followed by computation of 36 statistical parameters; on the basis of a stationary test 25 units were used for further analysis. The 6-OHDA treated group showed a significant greater time-dependency (i.e. deviation from a Poisson-process, as reflected by peaks in the spectrum of counts) as well as a stronger tendency towards temporal clustering of the action potentials and larger fluctuations in the firing rate from minute to minute. These results indicate that noradrenaline depletion may affect spontaneous neuronal activity patterns in the cerebral cortex.

The influence of the opiate receptor antagonist naltrexone on the central hypotensive action of alpha-methyldopa

P. L. M. van Giersbergen and W. de Jong, Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, NL-3521 GD Utrecht

The catecholaminergic system plays an important role in blood pressure regulation. Both peripheral as well as central administration of catecholamines results in marked cardiovascular changes, although of opposite direction. Injection of alpha-methylnoradrenaline, an active metabolite of alpha-methyldopa, directly into the nucleus tractus solitarii (NTS) caused a decrease in blood pressure. This decrease could be blocked by pretreatment with naloxone, an opiate receptor antagonist, suggesting involvement of endogenous opiate receptors. In fact beta-endorphin administered in low doses into the NTS caused a fall in arterial pressure. We further studied the role of the endogenous opiate system in the central hypotensive mechanism of alpha-methyldopa in conscious, normotensive Wistar rats. Alphamethyldopa produced a dose dependent fall in mean arterial blood pressure accompanied by bradycardia upon intracisternal administration. Pretreatment with naltrexone (125 µg/kg intracisternally) resulted in a parallel shift to the right of the dose response curve to alpha-methyldopa. In addition, the decrease in blood pressure after 0.5 mg/kg of alpha-methyldopa was inhibited in a dose dependent manner by increasing doses of naltrexone. These results indicate that the endogenous brain opiates are involved in the central cardiovascular effects of alpha-methyldopa.