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RENAL HEMODYNAMIC AND FUNCTIONAL CHANGES INDUCED BY INTERLEUKIN-1 IN THE RABBIT.

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Interleukin-1 (IL-1) may mediate several hemodynamic and renal changes observed during infection. The renal effects of iv recombinant IL-1-beta were studied in anesthetized and mechanically-ventilated rabbits, administered 0.5 (n=9) and 5 (n=8) µg/kg b.w. of iv IL-1. A rapid decrease in systemic blood pressure occurred in IL-1 treated rabbits, from 99.2±3.4 to 82.9±3.5 mmHg (0.5 µg/kg) and from 99.3±4.1 to 69.5±4.8 mmHg (5 µg/kg). Rectal temperature increased +1.85°C (0.5 µg/kg) and +0.85°C (5 µg/kg) at the end of the experimental period. Diuresis increased +41.9% (0.5 µg/kg) and +38.8% (0.5 µg/kg). Fractional sodium excretion (FENa) increased +79.6% (0.5 µg/kg) and +42.7% (5 µg/kg). FECl and FEK also rose. Inulin clearance fell -17.7% (0.5 µg/kg) and -34% (5 µg/kg). Renal blood flow increased +16.4% (0.5 µg/kg) and +18.1% (5 µg/kg), along with increased diuresis and sodium excretion. Filtration fraction rose and renal vascular resistance fell significantly. These results demonstrate that IL-1 induces hemodynamic changes similar to those observed in septic shock, associated with increases in renal blood flow, urine flow rate and sodium excretion, features also often observed during infection. In an additional experimental group of 5 rabbits, the injection of 100 ng of IL-1 in the left renal artery did not produce any change in diuresis and natriuresis in that kidney, nor in systemic blood pressure. These preliminary results suggest that the IL-1 does not *directly* affect water and sodium excretion.

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INTERLEUKIN-1β (IL-1β) AND TUMOR NECROSIS FACTOR α (TNFα) SYNERGISTICALLY INDUCE NERVE GROWTH FACTOR (NGF) SYNTHESIS IN RAT MESANGIAL CELLS

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Recent findings indicate that NGF, in addition to its neurotrophic function, also acts as an immunoregulatory cytokine. Thus, it was of interest to investigate whether inflammatory cytokines affect NGF production in mesangial cells which are involved in the control of immune function in the glomerulus. Our results show that the simultaneous addition of IL-1β and TNFα, but not of IL-1β and TNFα, elicited a marked (13-fold) increase of NGF protein released by cultured rat glomerular mesangial cells within 24 hr. This synergistic effect was dose-dependent (maximal at 1 nM) and due to enhanced gene expression since NGF mRNA of cytokine-treated cells was significantly elevated (5-fold) within 8 hr. Stimulation of NGF synthesis was abolished by mepacrine and dexamethasone, indicating that phospholipase A_2 may be involved in NGF regulation. These data suggest that a cytokine cascade including NGF may play an important role in the pathophysiology of inflammatory renal diseases.

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MODULATION OF NITRIC OXIDE (NO) SYNTHASE BY CYTOKINES IN RAT RENAL MESANGIAL CELLS (MC)

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Treatment of MC with interleukin 1β (IL-1β) or tumor necrosis factor (TNF) has been shown to induce NO synthase with subsequent stimulation of cGMP formation (Pfeilschifter & Schwarzenbach, 1990, FEBS Lett. 273, 185-187). Here we report that transforming growth factor β2 (TGFβ2) dose-dependently inhibits IL-1β- and TNF-stimulated cGMP formation in MC. Half-maximal inhibition was observed at concentrations of 0.3 ng/ml of TGFβ2. Maximum inhibition of cGMP formation over a 24 h period required the presence of TGFβ2 during the first 4 h of induction. In addition, the inhibitory effect of TGFβ2 on IL-1β- and TNF-induced cGMP formation is not affected by the potent cyclooxygenase inhibitor indomethacin, thus excluding prostaglandins as mediators.

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ETHINYLESTRADIOL (EE)-INDUCED CHOLESTASIS IS ASSOCIATED WITH INCREASED LIVER VOLUME, DECREASED SINUSOIDAL MEMBRANE SURFACE AND DESORGANIZED TIGHT JUNCTIONS

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Treatment of rats with EE, a potent synthetic estrogen, is an animal model for intrahepatic cholestasis of pregnancy in humans. In order to characterize and quantitate structural alterations livers from male SD rats (n=5 per group) treated with EE (5 mg/kg in 0.2 ml propylene glycol x 5 days, s.c.) or solvent alone were compared using standard stereological methods. EE induced significantly increased serum levels of alk Pase and bile acids but not of other markers. An increase in specific liver volume by +66% ($p<0.001$) was due to hypertrophy (volume of singular hepatocyte +35%, $p<0.001$) more than to hyperplasia (N_{vhep}/100g bw +23%, $p<0.001$). A decrease in sinusoidal membrane surface by 43% ($p<0.005$) was compensated for by the increased liver volume. In the canalicular tight junctions the number of strands was decreased from 4.7 to 3.8 (-19%, $p<0.005$). These data suggest that EE induces "mild" cholestasis characterized by increased junctional permeability and impairment of hepatocytes at the sinusoidal pole. Increased liver volume might reflect an adaptive response to compensate for the loss of sinusoidal membrane.

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CHANGES IN β_1- AND β_2-ADRENERGIC RECEPTOR mRNA LEVELS IN BROWN ADIPOSE TISSUE AND HEART OF HYPOTHYROID RATS

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The total density of plasma membrane β-receptors per tissue is decreased by 44 % in hypothyroid rat interscapular brown adipose tissue (IBAT) and by 55 % in hypothyroid rat heart. The densities of β_1- and β_2-AR per tissue are decreased by 50 and 48 %, respectively, in IBAT and by 52 and 54 % in the heart. Northern blot analysis of poly(A)$^+$ RNA from hypothyroid rat IBAT demonstrate that the levels of β_1- and β_2-receptor mRNA per tissue are decreased by 73 % and 58 % respectively, whereas in hypothyroid heart, only the β_1-receptor mRNA is decreased, by 43 %. The effect of hypothyroidism on the β_1-receptor mRNA is significantly more marked in the IBAT than in the heart. These results indicate that the decrease in β-receptor number in IBAT and heart of hypothyroid animals may in part be explained by a decreased steady state level of β-receptor mRNA.

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ARE THYROID HORMONES INVOLVED IN THE REGULATION OF TSH RECEPTORS IN THE THYROID GLAND ?

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This hypothesis was evaluated by determining the density of TSH receptors in 4 groups of rats : controls (C), hypothyroid (PTU treated), hypothyroid followed by T3 treatment (PTU + T3) and hyperthyroid (T3 treated). Hypothyroidism resulted in high TSH levels, low plasma and thyroid cellular T4, T3 concentrations (cT4, cT3). Saturation analysis of ^{125}I-bTSH to thyroid membranes showed two types of binding sites ($10^9 M^{-1}$ and $10^8 M^{-1}$). Hypothyroid rats vs C disclosed a 50 % reduction in the number of TSH binding sites of high affinity ($p<0.001$). PTU + T3 treatment did not modify the low TSH binding sites and the low cT3 content. Hyperthyroidism displayed high plasma T3, suppressed TSH, a moderate decrease in cT4 and cT3, but a 65% increased number of TSH binding sites of high affinity ($p<0.01$). In conclusion, the high TSH levels found in hypothyroid rats down regulate its own receptor. T3 treatment to these rats suppresses TSH secretion without changing the low TSH binding sites and cT3 concentration. The increased TSH binding sites associated with suppressed TSH in hyperthyroid rats suggest that thyroid hormones and likely thyroid T3 rather than plasma T3 concentrations may be involved in the regulation of TSH receptors.

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INSULIN SIGNALING AND CONTROL OF GLUCOKINASE GENE TRANSCRIPTION

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The glucokinase (GK) gene is the final target of two signal transduction pathways in the liver: i) insulin binding to its receptor leads to the activation of gene transcription, and ii) the glucagon/cAMP system represses GK gene transcription, an effect which overrides insulin action when both glucagon and insulin are applied together to cultured hepatocytes (J. Biol. Chem. 264, 21824-21829, 1989). Several inhibitors of the cAMP phosphodiesterases (PDEs), including isobutylmethyl-xanthine and RO 20-1724, have now been shown to prevent the insulin-dependent induction of GK mRNA. Interestingly, cGMP also inhibits the insulin effect. Activation of a cGMP-inhibitable form of PDE may therefore be an important factor in the mechanism of induction by insulin. We have further shown that okadaic acid, an inhibitor of protein phosphatases 1 and 2A, blocks the insulin effect. These data support the notion that increased protein phosphorylation by cAMP-dependent protein kinase is associated with gene repression, and that protein de-phosphorylation underlies the insulin-dependent induction of GK mRNA. In addition, continued protein synthesis appears to be a requirement for mRNA induction, as indicated by experiments using the protein synthesis inhibitors cycloheximide and anisomycin.

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APOPTOSIS IN MOUSE MAMMARY GLAND DEVELOPMENT.

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After lactating, the mouse mammary gland undergoes an epithelial cell apoptosis and tissue remodelling. The lactating mammary gland, composed of epithelium committed to milk protein synthesis, involutes to a quiescent organ composed predominantly of fat cells surrounding a denuded mammary epithelial "tree". Histological and immunofluorescence studies reveal the loss of epithelial cells concurrently with the transient appearance of apoptotic bodies and dramatic tissue reorganization. Southern blot analysis reveals non-random digestion of DNA. Northern blot analysis of RNA extracted from mouse mammary glands during involution shows a molecular signature of apoptosis: transient expression of specific genes involved in metabolism (LDH and ODC), stress response (heat shock protein 70), tissue remodelling (plasminogen activator proteins, collagenase and tissue inhibitor of metallo-proteases) and implicated in cell death (SGP-2).

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THE ENERGY COST OF LACTATION IN WOMEN LIVING IN THE GAMBIA

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The combined 24h energy expenditure of mother and child was measured with a respiratory chamber (indirect calorimeter) in a group of 16 lactating Gambian women and was compared to those of a control group of 16 non-pregnant non-lactating (NPNL) Gambian women. Breast milk production (738±47 g/24h) was adequate to allow a normal rate of growth of their two-month-old babies (28.0±2.4 g/24h). The energy retained by the child for growth in conjunction with the calorimetric measurements allowed to calculate the extra energy requirements for lactation, which were found to be 2100 kJ/day. These results confirm the values of the current dietary recommendations for lactation based on the energy cost of milk production.

Basal metabolic rate (BMR) was assessed under standardized conditions using a ventilated hood system. BMR of the lactating women averaged 3.807±0.063 kJ/min and was similar to that of the NPNL women (3.707±0.096 kJ/min) but these results were found to be 13% lower than values predicted on the basis of the FAO/WHO/UNU BMR standards. When the BMR's were normalized for fat-free mass, the lactating and non-lactating women had identical values, suggesting that the energy expended for milk synthesis is low.

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EFFECTS OF IV GLUCOSE-AMINOACID ON GLUCOSE METABOLISM IN LEAN HUMANS

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Glucose metabolism was studied on two occasions in 5 lean human subjects (age 37±(SD) 11 yrs, weight 64±8 kg) during a 3.5-hour glucose (11µmol/kg/min) infusion (G) followed by a 4-hour glucose + aminoacid (Vamina N, 32 µmol N /kg/min) infusion (AA). In study 1, U ^{13}C-glucose was infused to determine glucose turnover (GTO). In study 2, NaH^{13}CO3 was infused and neoglucogenesis (NG) calculated from ^{13}C plasma glucose enrichment.Insulin concentration increased from 96±(SD)41 pM after G to 130±44 after AA ($p<0.02$), and glucose concentration decreased from 6.7±0.5mM to 5.9±0.6 ($p<0.02$). AA increased glucose oxidation from 12.4±2.7 µmol/kg/min to 15.2±3.8 ($p< 0.002$), lipid oxidation from 0.94± 0.41 mg/kg/min to 1.02±0.45 ($p<0.05$), and aminoacid degradation from 4.2±2.2 µmol N /kg/min to 15.5±5.1 ($p<0.001$). GTO increased from 14.2±2.3 µmol/kg/min after G to 17.1±3.8 after AA ($p<0.02$), and NG increased from 1.2±0.6 µmol/kg/min to 3.9±1.6 ($p<0.01$).
Conclusions: 1) AA increases endogenous glucose production essentially by increasing NG; NG accounts for ca 70% aminoacid degradation. 2) AA stimulates glucose oxidation by increasing insulin secretion.

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A HISTOMORPHOMETRIC STUDY ON THE EFFECTS OF THE BISPHOSPHONATE CGP 42446 ON RAT BONE

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Histomorphometry of the rat proximal tibial metaphysis has been used to compare the skeletal effects of the new, highly potent bisphosphonate compound CGP 42446 [2-(imidazol-1-yl) -hydroxyethane-1,1-bisphosphonic acid] with those of the reference compounds pamidronate and etidronate.
Both pamidronate (10 nmol, 100 nmol, 10 µmol/kg/d) and CGP 42446 (0.1 nmol, 1.0 nmol, 100 nmol/kg/d) applied s.c. for 10 days induced a marked dose-dependent increase in the amount of newly formed mineralized cancellous bone. There was also a reduction in the osteoid perimeter in the pre-existing cancellous bone. The 2 compounds produced a small, significant decrease in longitudinal bone growth at the highest dose but the growth-plate thickness, and thus mineralization, were normal. At the highest dose of CGP 42446 trabecular number was significantly increased, reflecting a decrease in the physiological resorption of the mineralized cartilage septa. The concomitant reduction in both bone resorption and formation indicate an overall reduction in bone turnover. By contrast, almost no alterations were observed after etidronate treatment (10 µmol/kg/d).

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CELLULAR EFFECTS OF BDM IN SKELETAL MUSCLE

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Continued stimulation of frog muscle fibres gradually decreases twitch amplitude, eventually leading to rigor due to energy depletion. BDM (2,3-Butanedionemonoxime) reduces twitch amplitude within seconds in a dose dependent fashion to about 40% at 10 mM, but, in our hands, rigor never occured. Above 7.5 mM BDM we found in addition a use dependent decrease in excitability. The ATPase-activity of isolated myofibrils, actomyosin or even myosin alone is gradually reduced by over 50% by 1-30 mM BDM without affecting the Ca-sensitivity of the myofibrils. In isolated liver mitochondria from rabbit, the respiratory control rate (RCR), calculated from oxygen consumption, is similarly reduced. The RCR-depression is reversible upon washout or addition of 3µM CCCP, a decoupler of oxidative phosphorylation. The beneficial effects of BDM mainly seem to be do to the effect on the ATP processesing enzymes, synthetase and myosin. The reduced level of myoplasmic ATP resulting from inhibition of the synthetase may be affecting the plasma membrane properties. The depressed excitability and lower ATP-usage by moyosin seems to bring the muscle into a state of reduced activity protecting it against overexcitation.

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PATTERNED GROWTH OF NEONATAL RAT SKELETAL MUSCLE CELLS IN CULTURE

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Analysis of electrophysiological and polarization-optical signals of cultured skeletal muscle cells is limited by the fact that the cells grow irregularly and in random directions. A technique was used allowing to define the two dimensional arrangement and the orientation of the longitudinal axis of the cells. Conventional coverslips were coated with a UV-sensitive photoresist preventing cells to attatch and grow. Based on morphological and physiological criteria the coating didn't have toxic effects. The desired pattern from a high resolution film was projected to the coverslips. These were etched with a specific developer. Satellite cells were prepared and seeded with conventional culture techniques. The myocytes grew only in the groves etched out by the development procedure. They fused into myotubes of up to 15 mm total length in parallel strands along the desired pattern. Such a preparation is suitable for orientation sensitive optical experiments and to obtain defined neuro-muscular contacts in cocultures.

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MUSCLE ^{31}P-NMR SPECTROSCOPY DURING METABOLIC TRANSIENTS. POSSIBLE SOURCES OF BIAS.

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^{31}P-NMR spectroscopy can be used to assess phosphate (PC, ATP, Pi) levels in human muscles. The best time resolution with a 3T magnetic field is now on the order of 10 s. Thus it was possible to determine during graded submaximal rectangular loads on the plantar flexors : 1) the apparent kinetics of depletion and resynthesis of PC; 2) the apparent steady state [PC] vs power (w) relationship; 3) the time course of the Δ[PC]/Δ[Pi] stoichiometric ratio. The above values, however, might be subject to bias from changes of the pseudo first order chemical exchange rate constants K_1 (CPK) and K_2 (ATP-ase) of the muscle PC ATP Pi 3 site system, which could affect the area of the peaks. Therefore, a mathematical model was developed whereby the algorithms used for the calculation of the rate exchange constants were reversed in order to obtain from known K_1 and T_1 values the corresponding ^{31}P-NMR spectra. Whereas K_1 appears to be uninfluential, changes of K_2 within the physiological range may lead : a) to underestimate steady state exercise Ptot (=PC + ATP + Pi) and b) to alter the kinetics of [PC] splitting and resynthesis during the transients. Nevertheless, deviations of Δ[PC]/Δ[Pi] from unity may provide a qualitative index of changes of K_2.

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ENERGY SUPPLY DURING FLIGHT: A COMPARISON BETWEEN 3 SPECIES OF MIGRANT BIRDS

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Serum metabolites of the fat, protein and carbohydrate metabolism were measured in free-living Garden Warblers, Pied Flycatchers and Robins, which were caught during migratory flight. In order to define the metabolic responses specific to flight - a situation including activity and fasting - we compared the samples with those of overnight fasted, inactive birds. Birds during flight utilize fat, indicated by high FFA, glycerol and, suprisingly, triglyceride (TG) levels. It is hypothesized that the high TG levels point to another design of endurance energy supply to the muscles. Birds also utilize protein during flight, indicated by increased uric acid levels. In flying birds levels of all metabolites differed among species. The metabolic pattern of the Garden Warbler was characterized by a high fat catabolism, a low protein utilization and a low glucose level. Concomitantly, Garden Warblers, which winter South of the Sahara, cross Switzerland with the highest fat reserves. The Robins showed an opposite pattern relying less on fat. Wintering in the Mediterranean area they cross Switzerland with the lowest fat reserves of the 3 species.

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PHYSIOLOGICAL EFFECTS OF EXAMINATION STRESS IN STUDENTS: A REAL EXAMINATION VERSUS A LABORATORY SITUATION

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A preliminary study revealed a negative correlation of oral examination performance with anxiety, as well as a positive correlation of performance with increases in heart rate and systolic blood pressure.
The present study was aimed to compare a laboratory examination situation with a real exam, students were first tested in a video recorded lab session during the semester when they underwent a short oral exam. Heart rate was continuously assessed from the ECG, and blood pressure was measured every minute. Performance was evaluated offline, and high marks gave a bonus for the real exam. Further, subjective ratings of performance, stress feelings in every phase, and the preparation for the lab exam were assessed. To differentiate the physiological reactions with regard to active and passive coping behaviour, a cold pressor test was carried out at the end of the session as a reference. In the real examination situation, heart rate and blood pressure were measured before and after a written examination, which lasted 4 hours. Again subjective ratings, including anxiety and expected marks, were assessed before and after the test.

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CONTINUOUS RECORDING OF HEART RATE AND ACTIVITY IN SMOKERS AND NONSMOKERS UNDER FIELD CONDITIONS

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A new portable monitoring device was used to extend objective observations of physiological and behavioral parameters in smoking research, which so far have been limited to laboratory procedures, to field conditions. Heart rate was assessed from the ECG, activity with a built-in actometer and smoking periods with an event marker. Three experiments were carried out in order to evaluate the test-retest reliability of the devices, their potency to differentiate smokers from nonsmokers according to heart rate and/or activity measurements, and to investigate the effects of partial smoking abstinence on these parameters. All three experiments revealed a considerable test-retest reliability of the system. Female smokers differed from female nonsmokers in total heart rate but not in total physical activity. On the other hand, smoking abstinence during the first few hours in the morning resulted in a lower heart rate level compared to non-abstinent control conditions. These results suggested that the new system of continuous recording of heart rate, activity and smoking intervals might be useful in assessing smoking behavior and its physiological consequences under field conditions.

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HEART RATE VARIABILITY IN HUMAN CARDIAC TRANSPLANT RECIPIENTS DURING MODERATE EXERCISE. EVIDENCE FOR A POSSIBLE REINNERVATION ?

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Heart rate spontaneous fluctuations were measured by spectral analysis of RR-intervals in 9 cardiac transplant recipients (CTR) 4 to 34 months after the transplantation and in two control subjects in order to evaluate the autonomic nervous system activity to the heart. In controls, the spectra are divided into two frequency domains: low frequency (LF, ca. 0.1 Hz) due to both parasympathetic and sympathetic nervous systems, and fluctuations at the respiratory frequency (RF, ca. 0.25 Hz, known as the respiratory sinus arrhythmia) mediated by the parasympathetic only. RF fluctuations are reduced but not suppressed during moderate exercise (5 min at 75W). This indicates that the parasympathetic activity is not completely withdrawn. In most of the CTR, little or no heart rate variability was measured. However in two cases of long term transplantation (34 and 22 months) a pattern of variations similar to that of the controls was found. This latter finding may reflect either a mechanical effect, or the role of the recipient's atrial node, or more likely a reinnervation phenomenon.

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THE EFFICACY OF THE FRANK STARLING MECHANISM IN THE HUMAN TRANSPLANTED HEART

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At the onset of a constant-load exercise the heart rate (HR) response of heart transplant recipients (HTR) is delayed. The breath-by-breath VO_2 kinetics during the rest to work transient appears to be relatively fast ($t\frac{1}{2}$ = 50s), assuming a rate of readjustment of cardiac output (Q) as slow as that of HR. Therefore, on 21 HTR and 10 controls (CTL) we determined HR and the stroke volume of the heart (SV) on a beat-by-beat basis by impedance cardiography . At rest, in HTR (CTL) HR = 102 b/min ± 12 S.D. (72 ± 9); SV = 71 ml ± 26 (94 ± 16); Q = 7.2 l/min ± 2.4 (6.8 ± 1.5). At 50W, HR = 120 b/min ± 12 (98 ± 16); SV = 130 ml ± 39 (137 ± 50) Q = 15.6 l/min ± 5.3 (12.8 ± 3.6). The kinetics of readjustment of Q in HTR was only moderately slower than in CTL ($t\frac{1}{2}$ = 52 s ± 16 vs 40 ± 17). It is concluded that the rapid increase of Q found in HTR reflects essentially the quasi-instantaneous increase of SV that occurs within the second beat following work onset, the prove of the existence of a powerful Frank-Starling mechanism.

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A ROLE OF MITOCHONDRIAL CALCIUM TRANSPORT IN MYOCARDIAL HYPERMETABOLISM AFTER REPERFUSION.

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During postischemic reperfusion, myocardial oxidative metabolism may be exceedingly high compared to contractile function. To study the possible involvement of mitochondrial Ca^{++} transport in this phenomenon, ruthenium red (RR), an inhibitor of mitochondrial Ca^{++} transport, was added to the reperfusion medium in isolated rat hearts subjected to severe ischemia.
In untreated hearts (ISCH), after 15 min of reperfusion, developed left ventricular pressure (DLVP) was severely depressed to 7% of the values measured in nonischemic control hearts (CTR), but myocardial oxygen consumption (MO2) was similar (84%). RR treated hearts (ISCH-RR), showed a significant reduction of MO2 (54% of CTR) and an improvement of DLVP (26%). After 60 min of reperfusion, ISCH-RR hearts exhibited less damage indicated by improved recovery of DLVP (64% of CTR vs 18% in ISCH), an improved creatinephosphate content (86% vs 32% in ISCH) and a reduction by half of the loss of creatine kinase.
Thus, enhanced mitochondrial Ca^{++} transport is at least in part responsible for the dissociation between contractile function and oxidative metabolism after reperfusion.

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HIGH- AND LOWLANDERS AT ACUTE SIMULATED ALTITUDES.

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The respiratory, cardiovascular, and psychomotor reactions to acute hypoxic hypoxia were compared during a simulated exposure to high altitude in two groups of volunteers: residents of Zermatt (1616m) working at about 4000m and lowlanders (450m). The subjects underwent in supine position a standarized stepwise ascent to 6000m (P_B 354mmHg) in a low pressure chamber; the duration of the entire altitude exposure, apart from the adaptation time before ascent, was 2h. Results: The highlanders exhibited higher ventilation (the difference decreasing with altitude), at 6000m a lower heart rate x puls pressure product (calculated cardiac output) and a smaller difference between alveolar and transcutanous partial oxygen pressures as well as higher hemoglobin saturation values. The psychomotor function assessed with the d2-test (attentiveness) in the control subjects was better at the beginning of ascent but was, in contrast to the highlander group, reduced above 4000m. It is concluded that highlanders have more efficient ventilatory and more economic circulatory reactions which enable them to resist acute hypoxic hypoxia and to avoid central hypoxia better than lowlander controls.

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HYDROGEN PEROXIDE AN ENDOGENOUS SMOOTH MUSCLE CELLS HYPERPOLARIZING AND RELAXING FACTOR.

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Hydrogen peroxide (H_2O_2) can be released by different cells as the nerves, the endothelial cells and phagocytotic white blood cells. All these cells can interact with blood vessels wall. In the search for endogenous factors which relaxe vascular smooth muscles by hyperpolarizing their membranes, we address the question of a possible effect of H_2O_2 on the membrane potential and mechanical tension of pig coronary arteries smooth muscles. We show that 0,1 mM and 1mM H_2O_2 hyperpolarize these cells from 4 ± 0,6 mV (n=6) and 12 ± 1 mV (n=4) respectively during the relaxations this radical produces (cells membrane potential -49 ± 1 mV, n=10). We tested whether the endothelium derived hyperpolarizing factor (EDHF), released by the endothelium in response to bradykinin and substance P, is H_2O_2 by using catalase, an enzyme which hydrolyses H_2O_2. Catalase 4,000 U/ml suppresses the effects of exogenous H_2O_2, but it does not change the hyperpolarizations and relaxations caused by the kinins. We conclude that EDHF and hydrogen peroxide are two distinct molecules in this tissue.

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A23187 HYPERPOLARIZES ENDOTHELIAL AND SMOOTH MUSCLE CELLS OF PIG CORONARY ARTERIES

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SP and BK hyperpolarize and relaxe smooth muscle cells of pig coronary arteries in an endothelial-dependent manner. They also hyperpolarize coronary endothelial cells. The current idea to explain the endothelial cell hyperpolarization is that SP and BK induce an increase in cytosolic Ca^{2+} which activates K^+-channels. Therefore, we have used A23187, a Ca^{2+} ionophore, to see whether an increase in Ca^{2+} is responsible for this hyperpolarization. We show that 0.5µM A23187 hyperpolarizes endothelial cells from 20.0±1.7 mV (n=6) with a concomitant hyperpolarization (12.5±3.3 mV) and relaxation of the underlying smooth muscle cells (Em=51.5±1.2 mV and-44.6±2.4 mV, respectively). In order to see whether Ca^{2+}-activated K^+-channels are involved in the endothelial cell hyperpolarization, two blockers specific for these channels (apamin, charybdotoxin) and two more general K^+-channels blockers (TEA, 4-aminopyridin) were used. None of them inhibits electrical responses generated by A23187. Our results suggest that A23187 induces a hyperpolarization of pig coronary arteries endothelial cells, but this effect is not mediated by Ca^{2+}-activated K^+-channels, or these channels are not blocked by the antagonists used.

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NEWBORN RAT ARTERIAL SMOOTH MUSCLE CELLS MAINTAIN DIFFERENTIATING ACTIVITIES IN VITRO.

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Arterial smooth muscle (SM) development is characterized by an accumulation of α-SM actin and desmin (Kocher et al., Circ. Res. 56:829, 1985). The expression of these proteins decreases importantly during atheromathosis (Kocher et al., Lab. Invest. 50:645, 1984; Kocher and Gabbiani, Human Pathol. 17:875, 1986) and when smooth muscle cells (SMC) are placed in culture (Skalli et al., J. Submicrosc. Cytol. 18:481, 1986). In order to establish a model of in vitro differentiation, SMC were isolated from newborn rat aortic media and plated in 10 % FCS. After 7 days of culture, an important proportion of synthezised α-SM actin was maintained and double immunofluorescence staining showed an increase in the number of α-SM actin (anti-αSM-1) and desmin containing cells. α-SM actin mRNA expression was also increased at this time. Heparin (Grade II; Sigma Chemical Co, St Louis, MO) used to a final concentration of 200µg/ml (Desmoulière et al., Arteriosclerosis, In Press) increased α-SM actin content per anti-αSM-1 positive cell and the proportion of synthezised α-SM actin, but did not influence desmin expression. Northern blot analysis and in vitro translation of total RNA indicated that heparin acts at the translational or post-translational level. Thus, cultured newborn rat aortic SMC are capable to some extent of in vitro differentiation; heparin amplifies this phenomenon.

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Role of intussusceptive capillary growth in microvascular development of chicken chorio-allantoic membrane (CAM)

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Microvascular growth by intussusception represents a new mode of capillary network expansion. It has been described for the first time in the pulmonary capillaries (Caduff et al., 1986) and consists in the formation of transcapillary tissue pillars which transform into capillary meshes. It complements or substitutes the concept of capillary growth by sprouting.

Electron microscopic investigation of serial sections of the chicken CAM on days 5 and 7 of incubation indicates that its microvasculature seems to consist of a single flat sinus traversed by interspersed pillar-like structures and not of a meshwork of interconnected tubuli as proposed by Sethi and Brooks (1970). The observed pillars represent mostly simple zones of contact between upper and lower endothelial leaflets. Alternatively, the lumen is sometimes bridged by single thick endothelial cells squeezed between upper and lower sinus wall. On day 7, some pillars additionally contain a core of interstitial elements. Thus, pillar structure resembles the one observed in intussusceptive capillary growth in rat lungs (Burri and Tarek, 1990). These findings indicate that the CAM capillary system could initially be formed by intussusceptive microvascular growth.

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A NEW LUNG ORGAN CULTURE METHOD SUITED FOR OBSERVATION OF GROWTH FACTOR EFFECTS USING CONFOCAL MICROSCOPY

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Organ cultures represent a common compromise between the simplicity of cell cultures and the complexity of whole organisms. The advantage of this type of cultures is that they combine the preservation of the three-dimensional and topographical interrelations of cells in the living animal and the ease of handling of in vitro systems.
For lung explants different methods of culture have been described. We combined different elements from these methods and developed a novel, simple and inexpensive organ culture method, which in essence maintains the alveolar structure for several days. To this effect, lungs are instilled with a mixture of medium and agar, sliced and cultured on an inclined rotating plate.
Our interest focuses on growth effects of serum from pneumonectomized rats. The serum is believed to contain a lung specific growth factor responsible for the compensatory growth of injured lungs. Effects of such sera on our cultures will be monitored by confocal laser scanning microscopy.

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THE ROLE OF CENTRAL COMMAND FOR THE VENTILATORY RESPONSE TO EXERCISE

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In order to study the cortical component of the neural ventilatory drive, the so called central command, the ventilatory response to voluntary single leg exercise (i.e., with central command) and electrically induced exercise (i.e., without central command) was compared at different work loads. The experiments were carried out on 12 volunteers. In 6 different sessions one of the following exercise protocols was used at random: 5 min of rest followed by 15 min of isometric rhythmic exercise (alternating 4s contraction and 12s relaxation) either voluntary or electrically induced with work intensities of 5%, 15% or 25% of maximal voluntary contraction (MVC). The quadriceps muscle group was stimulated by a computerized muscle pocket exerciser (MediCompex SA). Force, ventilation, and oxygen uptake were measured continuously. At 15% and 25% MVC, our experiments revealed an increase in ventilation proportional to the increase in oxygen uptake independent whether the exercise was induced voluntary or electrically. At 5% MVC of voluntary exercise however, ventilation raised at a greater rate than oxygen uptake. It can be concluded that an adequate ventilatory response to exercise is possible without central command. Central command may even cause an overshoot of ventilatory response at low work loads.

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THE EFFECTS OF AIR POLLUTION ON LUNG FUNCTION OF SCHOOL-AGE CHILDREN

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In a longitudinal study lasting 12 months, the effects of air pollution (SO_2, NO_2, NO, ozone) on the lung function (VC, FEV_1, Raw, SGaw, $MEF_{50\%}$, $MEF_{25\%}$) of 33 healthy primary school children in Zurich were investigated. The pulmonary parameters of children from school exposed to high air pollution were not significantly different from those of children from the part of town with lower air pollution. However, the alterations of airway resistance (Raw) and specific conductance (SGaw) were parallel and significant in both groups: during the periods of increased NO_2, SO_2 or O_3 concentrations (October, February, July), Raw was increased and SGaw decreased. 24 out of the 33 children showed significant correlations between lung function and each of the environmental factors; 55 percent of children have at least two altered parameters correlating with those factors and are considered to be sensitive to air pollution.

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A MODEL FOR RETENTION OF INHALED PARTICLES DEPOSITED IN THE CILIATED AIRWAYS.

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Particles deposited onto surfaces of the ciliated airways are cleared rapidly by mucociliary activity. This clearance process is thought to be complete within 24-36 hours. The structural basis for an effective clearance from the ciliated airways might be the existence of a 2-phase system, consisting of a less viscous sol-phase that includes the cilia and above a more viscous gel-phase (mucus). The mucus may carry deposited particles towards the larynx by ciliary activity. In recent experiments particles were deposited in hamsters airways, where they were retained in close contact with the epithelium, between the cilia and not on top of the mucus, as expected. 24 hours later, 14% of these particles were still retained. We observed a surfactant bilayer between the gel- and sol-phases, and, from our surface tension measurements in sheep and horse tracheas we concluded that a surfactant film exists at the air-mucus interface. We hence propose the following model for the mechanism of particle retention in the ciliated airways: A particle deposited on the airway wall is displaced immediately into the mucus and eventually into the sol-phase by surface forces exerted on it by surfactant. From the sol-phase the particle will be cleared only slowly by phagocytosis of airway macrophages. Hence, a slow clearance process (days-weeks) must exist in the ciliated airways in addition to the established fast process.

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ULTRASTRUCTURAL ANALYSIS OF RELATIONSHIPS BETWEEN NUCLEOLAR STRUCTURE AND ACTIVITY

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Mouse spermatogenesis and early embryogenesis are suitable models for the study of the relationships between nucleolar structure and activity. The inactivating nucleoli in round spermatids and the reactivating ones in early embryos have been studied by means of cytochemical techniques (osmium ammine for DNA staining and Ethidium Bromide-PTA for nucleic acid visualization) and specific antibodies directed against DNA or RNA. Nucleoli undergoing inactivation in spermatids do not contain DNA but only a small amount of RNA, while in inactive spermatid nucleoli in cap phase nucleic acids are no longer detectable. On the other hand, DNA is present in the early embryo nucleolonema starting at the 4-cell stage, while RNA can be cytochemically detected already in the 2-cell stage inactive nucleolus. The relationship between nucleic acids and proteins related to the function and activity of the nucleolus will be discussed.

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TENASCIN SYNTHESIZED BY EMBRYONIC EPITHELIA AND PERIPHERAL GLIA ACCUMULATES IN THE SURROUNDING MESENCHYME

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The extracellular matrix protein, tenascin, has been proposed as mediator in epithelial-mesenchymal interactions in the embryo. Tenascin accumulates transiently in the dense mesenchyme surrounding growing epithelia, eg. lung buds. The protein is also expressed temporarily in the mesenchyme along peripheral nerve pathways. By in situ hybridization, we studied the cellular origin of tenascin in these cases. Tenascin mRNA was found to be accumulated exclusively by the bronchial epithelium at sites of active ingrowth. In developing peripheral nerves, tenascin mRNA is mainly produced by glial precursor cells. Thus, the protein seems to be accumulated in the mesenchyme at sites distant from its place of synthesis. Since tenascin can inhibit spreading of mesenchymal cells, it could cause retraction of mesenchyme along growing nerves and epithelia.

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VOLATILE ANESTHETICS AND LIVER BLOOD FLOW IN HUMANS

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Some volatile anesthetics are known to reduce hepatic blood flow in animals. Studies in humans are difficult because of the marked inter- and intraindividual variability of hepatic blood flow (HBF). In healthy patients undergoing minor surgery HBF was determined by indocyanine-green (ICG) under continuous registration of cardiac output (CO) by means of the noninvasive bioimpedance method. After a first i.v. bolus of 0.3 mg/kg ICG blood samples were withdrawn at 2,4,6, 8,10,12 and 14 min. A general anesthesia was induced with etomidat, fentanyl and suxamethonium chloride and maintained with either halothane or isoflurane (1 MAC) in O_2/air. At steady state, as verified by the endexpiratory concentration of the respective volatile anesthetic, usually 45 min after the first ICG application, a second bolus of 0.3 mg/kg ICG was given and blood samples were taken. ICG concentration in serum was determined photometrically at 800 nm. The ICG-clearance as marker of liver blood flow was calculated by the area under the curve (trapezoidal rule). Under halothane CO and HBF dropped to about 60% of the initial value whereas during isoflurane-anesthesia despite a decrease in CO the HBF was not altered.

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VOLATILE ANESTHETICS INCREASE THE THIOPENTAL-BINDING IN TISSUE-HOMOGENATES OF THE RAT

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It was reported that the thiopental-concentration in the tissue of Langendorff's heart preparation was increased if simultaneously halothane (H) was present (Experientia 1990:46:519). Question arose whether generally in the presence of a volatil anesthetic the binding of thiopental in the tissue was increased. The binding of thiopental (0.4 $mmol \cdot l^{-1}$) in the tissue homogenate of rats (liver, brain, heart, kidney, lung, spleen and skeletal muscle) was studied by equilibrium dialysis; beside H also enflurane (E) and isoflurane (I) were tested. % of thiopental bound was increased vs. control in the presence of H (11.8 $mmol \cdot l^{-1}$) in all tissues investigated at least to a factor of 1.4 (spleen) and maximally of 2.4 (brain). The same finding of an increased thiopental-binding, yet in a significantly lower extent as compared with H, was obtained if 10.3 $mmol \cdot l^{-1}$ E (except skeletal muscle) or 10.2 $mmol \cdot l^{-1}$ I (except kidney, spleen and skeletal muscle) were present.

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PHENOCOPY OF EXTENSIVE CARBOCYSTEINE METABOLISM BY ACETYL CYSTEINE CO-ADMINISTRATION IN POOR METABOLIZER SUBJECTS

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For the analysis of extensive (EM) and poor metabolizer (PM) phenotypes of carbocysteine (CC), 750 mg of CC are administered p.o. and urines are collected from 0-8 h and 8-16 h followed by TLC analysis. Using this modified CC protocol, deficient CC metabolism can be detected in 10 % of Swiss and British subjects. After co-administration of N- acetyl-cysteine (NAC, 1200 mg p.o.), we have carried out the same CC test with Swiss and British subjects using 4 EM and 4 PM cases of CC. In EM subjects, the results remained the same, whereas the PM phenotype is fully converted to the EM phenotype in the presence of NAC. When NAC metabolism was studied in the absence of CC, no NAC metabolites could be detected. Thus, it seems that the CC test provokes important inter-individual differences of organosulfur handling which can be counteracted by NAC administration. The common pathways of CC and NAC metabolism and the potential contributions by endogenous organosulfur compounds are unknown at this time. In summary: in the PM phenotype, the co-administration of NAC in the CC test is able to produce an EM phenocopy via the production of the same organosulfur compound present in EM urines after CC.