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Abstracts

MF A-1 Metabolism of neonatal respiratory neurons

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Isolated brainstem-spinal cord preparations of 0-4 day old rats are established models for the analysis of respiratory rhythm generation in the lower brainstem of neonatal mammals. Exposure to hypoxic solutions for up to 1 h produces tissue anoxia leading to an initial acceleration and a secondary slowing of the frequency of respiratory activity, similar to the biphasic hypoxia response of intact animals. In contrast to a decrease of extracellular pH (pH_e) by up to 0.5 pH units, extracellular K^+ activity (aK_e) only rises by about 1 mM and extracellular Ca^{2+} ($[Ca^{2+}]_e$) is not affected. Very similar responses can be evoked by application of cyanide. Blockade of anaerobic metabolism by iodoacetate produces a rapid and irreversible blockade of respiratory rhythm. A rise of aK_e by up to 30 mM and of pH_e by maximally 0.3 pH units is accompanied by a fall of $[Ca^{2+}]_e$ by more than 1 mM. The results indicate that anaerobic metabolism is sufficient for maintenance of respiratory activity in the neonate.

MF A-2 Dimension of diffusion space for intermediate transfer between membrane-bound enzymes

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Metabolic intermediate transfer processes are often facilitated in-vivo by enzyme aggregation and sequestration of enzymes and substrates in specific subcellular microenvironments. Testicular androgen biosynthesis is considered as a model for such a compartmentalized pathway; it comprises a first reaction sequence (cholesterol→pregnenolone(P)) catalysed within the mitochondria and a second reaction sequence (P→testosterone(T)) catalysed within the membranes of the smooth endoplasmic reticulum. The complete reaction sequence can be simulated by combining both isolated membrane fractions at various ratios and various final concentrations. Retention by the membrane compartment and secretion into the aqueous milieu is compared for all intermediates and products. Though P accumulates to a very high degree in the membranes, it is the only intermediate which is secreted in significant amounts (40% of T). In contrast, leakage of progesterone and 17-hydroxyprogesterone can be neglected: a large fraction (80%) of both intermediary steroids remain in the enzyme-bound state during steady-state conversion of the endogenous substrate cholesterol. Detailed analysis of this model system provides information on the consequences of three- vs. two- (or even nearly one-)dimensional diffusion for efficiency of intermediate transfer between enzymes of a metabolic pathway.

MF A-3 Vitality status of cells in multicellular spheroids

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We like to discuss the well-known model of "three distinct concentric annular shells" for cancer spheroids grown in a suspension culture with respect to new data given from optical sectioning with the aid of CLSM of spheroids loaded with fluorescence dyes. Cells of different growth activity may respond in their own characteristic fashion with a characteristic sensitivity to various fluorescent vital/lethal dyes. As a vital dye Fluoresceindiacetate (FDA) was used to detect the rim of proliferating cells and as lethal dye Lucifer Yellow VS as well as Lucifer Yellow CH were tested to mark the necrotic core. The evolution of the spheroids and the development of the necrosis can be described with these fluorescent markers. In the outermost shell cells are observed to grow and divide as they do in the initial exponential phase. In the adjoining shell, cells are alive and viable, but exhibit almost no mitosis and proliferation. Symmetrical distribution of FDA showed a decline of viable stained cells towards the centre at a distance of 40-100μm from the edge of the spheroid. In the innermost central core the cell membranes are ruptured and the tissue is in a state of disintegration. At a spheroid diameter of 350-450μm a central necrotic core appeares, intensively stained with Lucifer Yellow VS or Lucifer Yellow CH. This method represents a new approach to estimate the amount of viable and nonviable cells in multicellular spheroids, an important parameter to understand growth control and sensitivity to radiation in this tumor model.
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MF A-4 Selective oxidation of the main phospholipd from Thermoplasma acidophilum

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The main phospholipid (MPL) from the archaebacterium Thermoplasma acidophilum is a bipolar membrane-spanning tetraether lipid with a sugar component (presumably glucose) at one polar end and a glycerol phosphoester at the other polar side. Periodic acid was used at various concentrations for selective oxidation of either the vicinal hydroxyl groups at the glycerol moiety (12h, -25°C) or at both polar ends, i.e. the glycerol and the sugar components (RT). In the latter case, the sugar component was oxidized more rapidly than the glycerol. The reaction was controlled by thin layer chromatography and HPLC.
The purpose of these oxidative modifications is to introduce reactive structures into the lipid molecule capable of coupling reactions with separation media to achieve stable lipophilic surfaces.