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CADMIUM AS POWERFUL INHIBITOR FOR CA^{2+} AND PHOSPHATE TRANSPORT IN RESPIRING RAT LIVER MITOCHONDRIA (RLM).
R.Kiehl, Institut für Physiologische Chemie der Ruhr-Universität Bochum, D-4630 Bochum, FRG

Cd^{2+} has been reported to reversibly uncouple (stimulate oxidation and turn OS-ATPsynthetase into an OS-ATPase) in RLM at 2n atom/mg RLM, whereas concomitantly induced active swelling as well as Cd^{2+}-binding and accumulation could not be reversed. Above 4n atom/mg, Cd^{2+} inhibits respiration essentially from the inside by acting on complex III activity. Ca^{2+} and P_i-content of RLM are strongly decreased (90 and 80%) at essentially the same concentration stimulating oxidation and ATPase activity. Cd^{2+} does not have, as uncouplers do, any effect on isolated OS-ATPsynthetase activity. Cd^{2+}-induced active swelling is in size similar to the one induced by Ca^{2+} and P_i and about half the extent obtained with NSPM(20 nmol/mg), which blocks almost totally all SH-dependent transport activities (20 to 80 nmol/mg). The swelling is strongly inhibited (totally in the presence of Ca^{2+}) by ruthenium red (2 nmol/mg) at concentrations abolishing Ca^{2+}/P_i-induced swelling and to 50/40% Ca^{2+}/P_i-uptake. Swelling is slowed down by NEM (30 nmol/mg), which gives rise to contraction upon Ca^{2+}-addition (P_i-present) and inhibition of Ca/P_i-uptake to about 30%.Cd^{2+} alone partly reduces extent of NSPM induced swelling. Cd^{2+}/P_i-induced stimulation of RLM oxidation rate is prevented to about 90% (100%) in oligomycin (oli/ADP) treated RLM similar to the Ca^{2+}/P_i-induced one. It is concluded that Cd^{2+} binds at the high affinity Ca^{2+}-transport and the P_i/H^+-symport system in here triggering membran transition. The induced Cd^{2+}-effects are similar to the reported effects induced by diamide. NSPM also shares some effects with Cd^{2+} or diamide. The common denominator of these reactions may be the impariment of the SH/SS-balance by the SH specific reagents hindering membran transitions needed for active transport as well as for ATP synthetase activity.

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LIPOPHILIC GROUP TRAPPING REAGENTS AND "HIGH ENERGY COMPOUNDS" AS USEFUL PROBES FOR MEMBRAN ASSOCIATED FUNCTIONS

Kiehl, R., Institut für Physiologische Chemie der Ruhr-Universität Bochum, 4630 Bochum

I started some years ago to search for chemical reagents, which would be highly reactive and able to trap nondestructive as well as selective functional groups in lipophilic environment [1]. The idea was to get more insight into the chemistry of some membrane associated functions of mitochondria: energy transfer and energy utilization (ion transport and ATP synthesis). - One approach is the use of lipophilic compounds where lipophilic binding and chemical reactivity are combined. I synthesized sensitive reagents for electrophilic groups ($-X^+$, $-Y^{\delta-}\sim X^{\delta+}-$), the best one proved to be N-tert. butyl-N'-n-nonylthiourea (NBTU). NBTU is a powerful non-protonophoric uncoupler in mitochondria. The oxygen analog N-tert. butyl-N'-n-nonylurea (NBU) is ineffective. Compa ring studies with the H^+-transfer inhibitor dicyclohexylcarbodiimide (DCCD) reveals that NBTU is acting at the same site, in here blocking by a different mechanism energy transfer in the ATP synthetase. The change from inhibitor to uncoupler molecule depends on stepwise transition from one chemical function to another ($-N^{\delta-}=C^{\delta+}=N^{\delta-}-$, to $-NH-CO-NH-$, and $-NH-C^{\delta+}S^{\delta-}-NH-$). The most sensitive and selective probe for detection of membrane associated thiol-dependent transport activity is the lipophilic thiol reagent N'-[N"-n-nonyl-4-sulfamoylphenyl]-maleimide (NSPM). Phosphate-, adenine nucleotide-, as well as Ca^{2+}-transport activities are blocked with the compound by covalent modification of the involved proteins [2]. But one has to be careful: NSPM interacts also with high affinity nucleotide binding sites (NADH-DH, BOXY-DH, or ATP synthetase) by its structural similarity with the adenine moiety of the nucleotides (compa ring studies with the analog compound N'-acetyl-N"-n-nonylsulphanilamide, ANSA). - Another type of approach is the use of "high energy compounds". Very useful approved to be the energy transfer inhibitor picrylacetate (PA) [3]. PA is not only a highly reactive acetylating reagent but also very selective by its possibility of charge transfer interaction with other aromatic systems. It is able to trap functional groups involved in a chemical intermediate cascade from redox reaction to phosphorylation by functional group exchange or by transacetylation (with tyrosin). PA shifts the low steady state level of chemical intermediates toward the inhibitor trapped site. Beside these possibilities it is an useful reagent for amino group (lys) modification.

[1] Kiehl, R. and Bäuerlein E. (1976) FEBS Letters 72, 24.
[2] Kiehl, R. (1980) 1. EBEC, 113.
[3] Kiehl R., Akinpelu, O., Hoffmann-Posorske, E., and Kordt, S. (1982) 2. EBEC.

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