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Response of 3T3 Cells on Stimulation of Adenylate Cyclase by Forskolin, Studied by Multinuclear NMR Spectroscopy

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Summary: Stimulation of 3T3 cells by forskolin (30μM) led to an increase in cellular cAMP levels, as detected by ^{31}P-NMR, in both PCA extracts and living cells incorporated in basement membrane gel (BMG) threads. At the same time cAMP was exported in large amounts to the extracellular space. The in vivo experiments showed a decrease of more than 50% in both the PCr and ATP levels within 90 min of stimulation with forskolin. Reperfusion with fresh medium without the drug resulted in the full recovery of the phosphorus energy metabolism. A significant increase in phosphocholine (PC) was observed in ^{1}H and ^{31}P spectra of the PCA extracts concomitant to the rise in cAMP level. ^{13}C spectra of PCA extracts prepared after forskolin stimulation in the presence of [1-^{13}C]glucose indicated a serious inhibition of glycolysis, which was followed by a disordering of the phosphorus energy metabolism. Our results show a significant imbalance in the metabolic homeostasis during stimulation of 3T3 cells with forskolin.

Key terms: Forskolin, cAMP, inhibition of glycolysis, 3T3 cells, in vivo NMR.

Forskolin, which is widely used to study the role of cAMP in cell regulation, activates adenylate cyclase (AC) in a fast and reversible manner[1]. This leads to a significant increase in cellular cAMP levels in various cell types. cAMP is an ubiquitous intracellular mediator which regulates many reaction processes. It activates cAMP-dependent protein kinases and is cleaved into 5′AMP by 3′:5′-cyclic-nucleotide phosphodiesterases. Although long term effects induced by increased cAMP levels are known to include the inhibition of cell proliferation[2,3], it is not clear yet which of the forskolin-mediated changes in cell metabolism lead to the decreased cell division rate. Furthermore, the high levels of cAMP produced by forskolin-stimulated cells raise the question as to whether this is followed by changes in the energy homeostasis.

Enzymes:
Adenylate cyclase, ATP pyrophosphate-lyase (cyclizing) (EC 4.6.1.1);
Diacylglycerol cholinephosphotransferase, CDPcholine:1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2);
Choline kinase, ATP:choline phosphotransferase (EC 2.7.1.32);
Choline-phosphate cytidyltransferase, CTP:choline-phosphate cytidyltransferase (EC 2.7.7.15);
3′,5′-Cyclic-AMP phosphodiesterase, 3′,5′-cyclic-AMP 5′-nucleotidohydrolase (EC 3.1.4.17);
Phosphofructokinase, ATP:D-fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.11);
6-Phosphofructo-2-kinase, ATP:D-fructose-6-phosphate 2-phosphotransferase (EC 2.7.1.105);
Phospholipase C, phosphatidylcholine cholinephosphohydrolase (EC 3.1.4.3);
Phospholipase D, phosphatidylcholine phosphatidohydrolase (EC 3.1.4.4).

Abbreviations:
AC, adenylate cyclase; AMP, adenosine phosphate; ATP, adenosine triphosphate; BMG, basement membrane gel; cAMP, adenosine 3′:5′-cyclic monophosphate; CDP, cytidine diphosphate; CTP, cytidine triphosphate; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediaminetetraacetic acid; FCS, foetal calf serum; NMR, nuclear magnetic resonance spectroscopy; PBS, phosphate-buffered saline; PC, phosphocholine; PCA, perchloric acid; PCr, phosphocreatine; PME, phosphomonoester.