THE CONTROL OF sn-1,Z-DIACYLGLYCEROL LEVELS IN GROWTH FACTORS AND NEUROTRANSMITTERS FREQUENTLY LEADS TO AN INCREASE IN DIACYLGlycerol CONCENTRATION. THIS INCREASE HAS OFTEN BEEN OBSERVED TO BE BIPhasIC, SUGgESTING THAT THE LIPID MESSENGER IS PRODUCED FROM MORE THAN ONE SOURCE. STRUCTURAL ANALYSIS OF THE DIACYLGlycerol FORMED IN CELLS HAS DEMONSTRATED THAT THE INITIAL PHASE CONSISTS OF POLYUNSATURATED DIACYLGlycerol SPECIES WHICH ARE PROMINENTLY DERIVED FROM INOSITOL PHOSPHOLIPID HYDROLYSIS. THE LATER PHASE CONTAINS AN INCREASE IN POLYUNSATURATED, MONounsaturated AND Saturated DIacylglycerol SPECIES. MANY OF THESE SPECIES ARE APPARENTLY DERIVED FROM PHOSPHATIDYLCHOLINE HYDROLYSIS. DIACYLGlycerol IS NOT JUST GENERATED BY PHOSPHOLIPASE C ACTION, IT IS ALSO GENERATED AS A RESULT OF A PHOSPHOHYDROLASE CATALYSED HYDROLYSIS OF PHOSPHATIDYCHOLINE. SPECIES ANALYSIS OF THE GENERATED PHOSPHATIDYLCHOLINE SPECIES HAS DEMONSTRATED THAT THE ACRYL PHYTANAE WHOSE CHAIN IS SATURATED OR MONounsaturated AND Saturated DIacylglycerol SPECIES ARE SATURATED OR MONounsATURATED. THEREFORE, THE DIACYLGlycerol GENERATED THROUGH THIS PATHWAY WILL BE SATURATED OR MONounsATURATED RATHER THAN POLYunsATURATED. IT IS PROBABLE THAT IT IS ONLY THE POLYunsATURATED DIacylglycerol SPECIES WHICH ARE ACRYL PHYTA WHOSE CHAIN IS SATURATED OR MONounsaturated AND Saturated DIacylglycerol SPECIES. DIACYLGlycerol IS REMOVED FROM CELLS BY A NUMBER OF PATHWAYS. OF MAJOR IMPORTANCE IS THE DIACYLGlycerol KINASE CATALYSED PATHWAY. CELLS CONTAIN A NUMBER OF ISOFORMS OF DIACYLGlycerol KINASE, OF PARTICULAR INTEREST IS THE IDENTIFICATION OF AN ISOFORM WHICH IS SPECIFIC FOR POLYunsATURATED DIacylglycerol SPECIES. THIS ENZYME HAS BEEN PURIFIED FROM THE MEMBRANES OF PORCINE TESTES AND DEMONSTRATES AN ABSOLUTE SPECIFICITY FOR POLYunsATURATED SPECIES. THE RESULTS WILL BE DISCUSSED IN RELATION TO THE DIFFERING ROLES OF DIFFERENT FORMS OF PHOSPHATIDYLCHOLINE AND DIACYLGlycerol AS MESSENGER MOLECULES AND THE REGULATION OF THE PATHWAYS OF THEIR REMOVAL.

THE REGULATION OF INOSITOL TRISPHOSPHATE BY INOSITOL POLYPHOSPHATE 5-PHOSPHATASES. C.A. Mitchell. Department of Medicine, Monash Medical School, Box Hill Hospital, Nelson Rd, Victoria, Australia, 3128

AGONIST-INDUCED HYDROLYSIS OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PtdIns(4,5)P2) RESULTS IN THE GENERATION OF THE CALCIUM MOBILISING INTRACELLULAR SECOND MESSENGER, INOSITOL 1,4,5-TRISPHOSPHATE (Ins1,4,5P3). THE INOSITOL POLYSphatase 5-PHOSPHATASES ARE AN EMERGING FAMILY OF ENZYMES THAT HYDROLYSE THE 5-POSITION PHOSPHATE AND THEREBY INACTIVATE INS1,4,5P3. AT LEAST TWO DISTINCT CLASSES OF PHOSPHATASE ENZYMES HAVE RECENTLY BEEN IDENTIFIED. THE TYPE I PHOSPHATASES ARE OF SMALLER MOLECULAR WEIGHT (APPROXIMATELY 43 KDa) AND HYDROLYSE AND INACTIVATE ONLY INS1,4,5P3 AND INOSITOL 1,3,4,5-TETRISPHOSPHATE (Ins1,3,4,5P4) WITH HIGH AFFINITY. THE TYPE II PHOSPHATASES HAVE A LARGER MOLECULAR WEIGHT (75-160 KDa) AND DECREASED AFFINITY FOR BOTH INS1,4,5P3 AND INS1,3,4,5P4 COMPARED TO THE TYPE I ENZYMES. IN ADDITION, THE TYPE II PHOSPHATASES HYDROLYSE POLYPHOSPHATIDES INCLUDING PtdIns(4,5)P2 AND PtdIns(3,4,5)P3. THE RECENT CLONING OF SEVERAL NOVEL 5-PHOSPHATASE ENZYMES HAS DEMONSTRATED THAT BOTH TYPE I AND II 5-PHOSPHATASES CONTAIN A CONSERVED, APPROXIMATELY 150 AMINO ACID DOMAIN, THAT MAY REPRESENT THE CATALYTIC OR SUBSTRATE BINDING SITE.

WE HAVE RECENTLY CLONED A 43 KDa 5-PHOSPHATASE USING AN ANTISENSE STRATEGY IN NORMAL RAT KIDNEY (NRK) CELLS. CELLS WITH DECREASED 43 KDa 5-PHOSPHATASE DEMONSTRATED A 2-4 FOLD INCREASE IN INS1,4,5P3 AND INS1,3,4,5P4, RESPECTIVELY, ASSOCIATED WITH A 1.9 FOLD INCREASE IN BASAL INTRACELLULAR CALCIUM LEVELS. CELLS UNDEREXPRESSIONING THE ENZYME DEMONSTRATED A TRANSFORMED PHENOTYPE AND FORMED TUMOURS IN NUCLEAR MOUSE. THESE STUDIES SUGGEST THAT THE REGULATION OF INS1,4,5P3, THE PHOSPHATASE INDICATES A 412 AMINO ACID PROTEIN AND A C-TERMINAL FRANESYLLATION SITE CVVQ. THE PHOSPHATASE IS KNOWN TO BE PRESENT IN RAT CEREBELLAR PURKINJE NEURONS. THE PROTEIN SEQUENCE DEDUCED FROM cDNA ANALYSIS SHOWS THE PRESENCE OF CONSERVED RESIDUES WITH OTHER MEMBERS OF THE INOSITOL PHOSPHATASE FAMILY PARTICULARLY A PROTEIN INVOLVED IN LOWE'S SYNDROME AND THREE GENES OF SACCHAROMYCES CEREVISIAE WITH UNIDENTIFIED FUNCTION. TWO ARG RESIDUES OF TYPE I INS1,4,5P3-5-PHOSPHATASE HAVE NOW BEEN IDENTIFIED AS TWO ACTIVE SITE RESIDUES DIRECTLY INVOLVED IN SUBSTRATE BINDING. IN ADDITION TO TYPE I INS1,4,5P3-5-PHOSPHATASE, BOVINE BRAIN HOMOGENATES ALSO SHOW THE PRESENCE OF TWO PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE 5-PHOSPHATASE ACTIVITIES.

REGULATION OF THE ADIPOCYTE cGMP- INHIBITED PHOSPHODIESTERASE (PDE3B) BY INSULIN- AND ISOPROTERENOL-STIMULATED PHOSPHORYLATION. Tova Rahn and Eva Degerman, University of Lund, Sweden, and Vincent C. Manganiello, NIH, Bethesda, MD.

Two cyclic GMP-inhibited phosphodiesterase (cGMP-PDE) isoforms, PDE3A and B, have been identified and cloned. PDE3A and B are products of different but related genes located on human chromosomes 12 and 11, respectively. PDE3B forms have been cloned from adipocyte and lymphocyte cDNA libraries; PDE3A, from cardiac and aortic smooth muscle libraries. In rat adipocytes, activation of a microsomal PDE3B is important in the antilipolytic action of insulin, which is blocked by specific PDE3 inhibitors. Insulin-induced activation of an intracellular serine kinase (ISK) results in phosphorylation [on Serine(S) 302] and activation of the adipocyte PDE3B. Wortmannin, a potent inhibitor of phosphatidylinositol-3-kinase (PI3-K), blocks the antilipolytic action of insulin as well as insulin-induced activation of ISK and phosphorylation/activation of PDE3B. This suggests that the insulin antilipolytic signal chain, including downstream components such as ISK and PDE3B, is regulated via receptor-mediated activation of PI3-K. PDE3B S302 is also phosphorylated in the presence of insulin and isoprotenerol, where insulin exerts its antilipolytic effect, phosphorylation of S302 is more than additive, suggesting that "crosstalk" between insulin- and CAMP-signalling pathways upstream of PDE3B regulates phosphorylation/activation of the enzyme and the antilipolytic action of insulin.