The amyloid precursor protein (APP) can be processed via several alternative processing pathways. Alpha-secretase processing by cleavage within the amyloid beta-peptide domain of APP is highly regulated by several external and internal signals including G protein-coupled receptors, protein kinase C and phospholipase A2. Stimulation of m1 and m3 muscarinic acetylcholine receptors (mAChR), which are coupled to phosphoinositide hydrolysis and protein kinase C activation, has been shown to increase the release of soluble amyloid precursor protein (αAPPs). There have been several reports indicating that Gq protein-coupled receptors including mAChRs (m1, m3), metabotropic glutamate receptors, and bradykinin receptors, regulate αAPPs secretion. However, there are no direct evidence for the exact roles of G proteins. In the present study, to examine the regulation of Gq protein-linked muscarinic receptor-mediated αAPPs release, we transiently transfected the different Gα carboxyl-terminal peptide (Gαq, Gαi), which have shown a novel dominant-negative strategy (Gilchrist et al.), in SH-SY5Y cells expressing abundant m3 muscarinic receptors endogenously. In wide type cells, increase in αAPPs released by normal metabolism of APP was detected in control medium in a time-dependent manner, and the αAPPs release was stimulated by carbachol, a muscarinic agonist, and phorbol 12-myristate 13-acetate (PMA), a PKC activator. The carbachol-induced increase in αAPPs release was blocked by EGTA, a Ca2+ chelator, indicating a Ca2+-dependent mechanism. On the other hand, PMA-induced αAPPs releases was Ca2+-independent. Furthermore, to examine the regulation of αAPPs secretion by upstream cellular signals, dominant-negative Gα carboxy-terminal peptide-expressing SH-SY5Y cells were examined, and the results are discussed.

[PA1-42] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

The role of intracellular Ca2+ increase and prooxidant production in the expression of ferritin light chain by sulfur amino acid deprivation in hepa1c1c7 cells

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Sulfur amino acid deprivation (SAAD) induces oxidative stress through depletion of glutathione content. Ferritin synthesis increases in response to oxidative stress conferring resistance to subsequent insults. However, the molecular mechanisms for the expression of the ferritin gene by oxidative stress have not been studied yet. In the present study, change in intracellular calcium content was determined as part of the complete studies on the expression of ferritin light chain (FLC) gene by SAAD in hepa1c1c7 a murine hepatoma cell line. Confocal microscopy showed that intracellular calcium level was 1.5-fold increased after SAAD up to 80 sec, which extended for the next 200-300 sec, followed by returning to control level. The elevation of calcium by SAAD was prevented by GSH, methionine, cystine or cysteine, indicating that change in the redox-state might control the cellular calcium level. Furthermore, either verapamil or thapsigargin was active in inhibiting the increase in cellular calcium by SAAD, raising the notion that the calcium increase by SAAD might result from the influx of calcium via Ca2+ channel as well as the release from endoplasmic reticulum. SAAD increased the oxidation of dichlorofluorescin. Treatment of cells with verapamil or deferoxamine, or deficiency of extracellular calcium prevented prooxidant production by SAAD. Hence, elevation of intracellular calcium by SAAD was responsible for the oxidative stress. Northern blot analysis revealed that SAAD increased the mRNA level of FLC, which was inhibited by either EGTA or deferoxamine. Taken together, these results provided evidence that increases in intracellular calcium and oxidative stress by SAAD might lead to the enhanced expression of FLC mRNA in Hepa1c1c7 cells.

[PA1-43] [10/19/2000 (Thr) 10:00 - 11:00 / [Hall B]]

The changes of catecholamines and indolamines of rat brains by extremely low