

streams of PLA2 pathways, COX and lipoygenase(LOX) pathways, are regulate the muscarinic-mediated sAPP release, we examined the effects of COX and LOX inhibitors on sAPP release. The COX and LOX inhibitors partially increased constitutive sAPP release, but failed to show significant change in oxoM -stimulated sAPP release. The COX and LOX inhibitors only reduced arachidonic acid induced sAPP release. Although COX and LOX are maybe involved in constitutive release of sAPP, our results indicated that muscarinic receptor mediated sAPP release is regulated by the generation of arachidonic acid through PLA2 activation rather than COX and LOX activities.

[PB3-5] [ 04/18/2003 (Fri) 09:30 - 12:30 / Hall P ]

### **Dihydropyrimidinase related protein-2 expression in focal ischemic rat brain and hypoxia-induced PC12 cell**

Chung Myung Ah<sup>o</sup>, Kim Hwa Jung

Collage of Pharmacy, Ewha Womans University

Ischemia-induced changes in protein expression may provide important insights into the mechanisms of cellular damage and their potential recovery. In the present study, to investigate protein patterns changed in ischemic condition, the cortical and striatal tissue samples from the permanent and transient ischemic rat brain obtained by middle cerebral occlusion were analysed by proteomic approach using 2D-PAGE and MALDI-MS. Among proteins shown to clear differences in their expression level, the dihydropyrimidinase related protein-2 (DRP-2) was identified in produce under ischemic condition, and therefore, DRP-2 might be a candidate new molecule that could play an important role in brain ischemic mechanism. The DRP-2 is known to be equivalent to TOAD-64(TUC-2), CRMP-2 and Ulip-2. Changes of expression level of this protein in ischemic brain were confirmed by western blotting using monoclonal DRP-2 antibody (C4G). The C4G antibody labeled three separated bands. Unexpectedly, the major band (64 kDa) and the upper band (66 kDa) with less density were decreased but the lower band (62 kDa) was clearly increased in their intensity according to the ischemic duration in both cortical and striatal region of the MCA-occluded rat model. Ischemia is commonly known as a condition of glucose and oxygen deprivation. Therefore, we tried to investigate whether the DRP-2 exprssion level also changes in hypoxia- induced PC12 cell, and obtained the similar pattern to that of the MCA-occluded rat model. While the 64 kDa band decreased faint gradually, the 62 kDa band was increased. Furthermore, these results were observed according to hypoxic duration and media condition which was absence or presence of glucose and serum. It can be postulated that the differentially changed three bands observed in ischemic rat brain and hypoxia-induced PC12 cell by DRP-2 antibody represent distinct isoforms of DRP-2 generated as result of alternative mRNA splicing, proteolysis, or its post-translationally modified DRP-2. The possibility of proteolysis and alternative splicing of DRP-2 mRNA during ischemic condition is being tested. In conclusion, at least three isoforms exist, and their expression level changed differentially in ischemic condition of rat brain and hypoxia-induced neuronal cells. Although the role of each isoform of this protein is still not known exactly, our results suggest the involvement of DRP-2 in ischemic and hypoxic condition, and these could be provided useful information to elucidate pathophysiological mechanisms and therapeutic strategies of stroke.

[PB3-6] [ 04/18/2003 (Fri) 09:30 - 12:30 / Hall P ]

### **Characterization of choline transport in immortalized rat brain capillary endothelial cell lines (TR-BBB)**