neuronal cells was investigated in this study. PC12 cells were incubated in the medium loaded with [3H]dopamine (0.5μCi/ml) for 3 h at 37℃ and then were incubated in Krebs-Ringer-HEPES buffer containing test drugs and HRF for 20min. The amount of dopamine release was determined by measuring radioactivity of media samples. Intracellular calcium ($[Ca^{2+}]i$) were determined by monitoring fura-2 fluorescence by the dual wavelength method. rHRF evoked dopamine release in a concentration- and a time-dependent manner, and also increased $[Ca^{2+}]i$ in a $Ca^{2+}$-containing buffer. rHRF did not produced a increase of $[Ca^{2+}]i$ in the absence of extracellular $Ca^{2+}$, however, interestingly, rHRF evoked dopamine release in the $Ca^{2+}$-free buffer, both dopamine release and $[Ca^{2+}]i$ increased by KCl and bradykinin were blocked in a $Ca^{2+}$-free buffer. Both dopamine release and $[Ca^{2+}]i$ increased by rHRF was not affected by a treatment of nifedipine (5 uM), a L-type $Ca^{2+}$ channel blocker, whereas dopamine release and $[Ca^{2+}]i$ evoked by KCl was inhibited. HRF-stimulated dopamine release was also not inhibited by a MAP kinase inhibitor, , or a calcium-dependent cPLA2 inhibitor. Only a selective inhibitor of calcium-independent iPLA2 produced an inhibitory effect on rHRF-induced dopamine release. These results suggest that rHRF-induced increase in dopamine release is controlled by the Ca2-independent process, and a $Ca^{2+}$-independent PLA2 pathway is involved in a HRF-induced dopamine release.

[PA1-64] [ 10/18/2002 (Fri) 09:30 - 12:30 / Hall C ]

## Pre-conditioning attenuated the $MPP^+$-induced cytotoxicity

Lee Seung-Jin°, Yang Sang-In, Jang Choon-Gon, Lee Seok-Yong

Lab. of Pharmacology, College of Pharmacy, Sungkyunkwan University

$MPP^+$ is known to be a neurotoxic substance that induces the degeneration of dopaminergic neurons and Parkinson-like syndrome. Incubation with $MPP^+$ induced the expression of heme oxygenase-1 (HO-1) in PC-12 cells and HO-1 revealed a protective effect against $MPP^+$-induced cytotoxicity. In this study, we tested the effect of pre-conditioning on the $MPP^+$-induced cytotoxicity. The PC-12 cells were incubated with $MPP^+$ for 3 hrs, and then after 12 hrs the cells were exposed to several concentration of $MPP^+$. Pre-incubation (pre-conditioning) with $MPP^+$ significantly attenuated the cytotoxic effects of $MPP^+$ and induction of heme oxygenase may be involved in this protective effect.

[PA1-65] [ 10/18/2002 (Fri) 09:30 - 12:30 / Hall C ]

## Action of lysophosphatidylcholine in U937 human monocytes

Yun Mi-Ran°, Im Dong-Soon

Laboratory of Pharmacology, College of Pharmacy, Pusan National University

Atherosclerosis is a main cause of cardiovascular diseases (that is angina, hypertension, cardiac infarction) and stroke. High level of low-density lipoproteins (LDL) in blood has been implicated as an important factor of atherosclerosis progression. Recently researches in endothelial cells unveiled the roles of lysophosphatidylcholine (LPC), a constituent of oxidized LDL in atherosclerosis. However, action of LPC in monocytes has not been studied. We challenged a set of LPC in U937 human monocytes and found that LPC stimulated cell growth and mobilized $Ca^{2+}$. The $Ca^{2+}$ response was not blocked by pertussis toxin, an inhibitor of $G_{i/o}$ proteins or U73122, a phospholipase C inhibitor. Furthermore, The response was totally blocked by EGTA addition in extracellular media, suggesting

that $Ca^{2+}$ influx across the plasma membrane was the only source of LPC $Ca^{2+}$ response in U937 cells. 16:0 and 18:0 LPC induced similar response in $Ca^{2+}$ mobilization. However, 16:0 only induced increase of [$^3$H]thymidine incorporation in this cell line. Recently a couple of G protein-coupled receptors were suggested as LPC receptors. However, our data suggest that LPC-induced responses in monocytes may not be mediated through G protein-coupled receptors and also that other action mechanism of LPC may be involved in the LPC responses, especially cell proliferation, in U937 cells.

[PA1-66] [ 10/18/2002 (Fri) 09:30 - 12:30 / Hall C ]

## Direct and functional interaction between dopamine D2 receptor and ALY

Yang JiHye°, Cheong DaWoon, HyungJu Seo, Kim MoonSoo, Kim Kyeong-Man

Pharmacology lab, College of Pharmacy, Department of Psychology (KMS), Chonnam National University, Kwang-Ju, 500-757 Korea

The signaling pathway of D2 dopamine receptor was studied using yeast two-hybrid system. The 3rd cytoplasmic loop of rat D2 dopamine receptor was used to screen the cDNA library of mouse brain, and ALY was found to interact with it. The interaction in the yeast was observed only with the 3rd cytoplasmic loop of D2 dopamine receptor but not with that of D3 or D4 dopamine receptor. The interaction between two proteins was also confirmed by GST pull-down assay. Co-expression of D2 dopamine receptor abolished ALY-induced enhancement of Lef-1 promoter expression in HEK-293 cells. In constrast, when cells were transfected with wnt-1 and dishevelled, ALY and D2 dopamine receptor synergistically enhanced the wnt signaling.

[PA1-67] [ 10/18/2002 (Fri) 09:30 - 12:30 / Hall C ]

## Comparative Studies of Molecular Mechanisms of Dopamine D2 and D3 Receptors for the Activation of Extracellular Signal Regulated Kinase 1/2 in HEK-293 cells

Cheong DaWoon°, Cho ChoonSil, Kim HyunJu, Kim Kyeong-Man

Pharmacology lab, College of Pharmacy, Chonnam National University, Kwang-Ju, 500-757 Korea

Dopamine D2 and D3 receptors (D2R and D3R) belong to pharmacological D2R family and share similar structural and functional characteristics. Elucidation of their differential functional characteristics is important for understanding their roles in brain. ERK1/2 was chosen as an example of signaling component of D2R and D3R and systemic studies were conducted to understand the regulatory mechanisms on ERK1/2 activation. Agonist-stimulated D2R and D3R induced ERK1/2 activation reached a maximal response at 5 min in a concentration-dependent manner. Haloperidol and sulpiride, antagonists of D2 like receptors, effectively attenuated D2R- and D3R-mediated ERK1/2 activation. Pertussis toxin abolished both D2R- and D3R- mediated ERK1/2 activation, suggesting that either Gi or Go type of G proteins is involved. Dominant negative mutant of dynamin (K44A) blocked ERK activation induced only by D2R but not by D3R. Both D2R and D3R-mediated ERK1/2 activation were attenuated by wortimannin, a inhibitor of phosphatidylinositol 3-kianse, Go6983, a PKC isotype-specific inhibitor. Interestingly, tryphostin AG1478, a selective inhibitor of tyrosine kinase activity in epidermal growth factor receptor and DN-Raf, a dominant negative mutant of p74raf-1 effectively blocked D2R-mediated ERK1/2 activation, but not that mediated by D3R. These results suggest that D2R activates ERK1/2 through the classical ERK1/2 cascades including transactivation with EGFR, however D3R uses distinct signaling pathways for the ERK1/2 activation.

[PA1-68] [ 10/18/2002 (Fri) 09:30 - 12:30 / Hall C ]